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71-14,243

McATEE, Joyce Wood, 1939-
DIETARY PROTEIN AND LIPID METABOLISM IN REFED RATS.

Iowa State University, Ph.D., 1970
Health Sciences, nutrition

University Microfilms, A XEROX Company, Ann Arbor, Michigan

DIETARY PROTEIN AND LIPID METABOLISM IN REFED RATS

by

Joyce Wood McAtee

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Nutrition

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1970

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INTRODUCTION

It has been amply documented that rates of lipogenesis may be greatly influenced by variations in the energy source of the diet in young animals recovering from short periods of starvation. However only a few studies have dealt with the response of chronically malnourished, adult rats to nutritional rehabilitation.

In a series of investigations in this laboratory the response in lipid metabolism to various dietary manipulations following severe weight reduction was assessed. For these studies an experimental model was developed in which a heightened state of lipid synthesis was established in adult rats. Changes in lipogenic capacity of hepatic and adipose tissue caused by variations in the dietary energy source were evaluated by body fat accumulation and alterations in tissue fatty acid patterns (Noble, 1967; Stadler, 1969). Protein in these diets had been low, approximately 4% of calories. Under these conditions the chronically starved adult rat responded to variations in dietary fat in the refeeding regimen much like the young animal subjected to acute starvation. In subsequent experiments, where protein nutriture was also varied, (Lee, 1967) it became apparent that other parameters related to lipogenesis, such as activity of NADPH producing hepatic enzymes, might be modified by protein intake. With excessive levels of dietary protein a depression occurred in the activ-

ity of the hexose monophosphate shunt dehydrogenases (HMPD), though overall rate of recovery of lipid stores was as good or better with high as with low protein intakes. Formerly this enzyme system was thought to be well correlated with rate of lipogenesis. More recent studies indicate that shunt activity and incorporation of labelled substrates into fatty acids may not always be correlated (Tepperman et al., 1968).

The study reported here was designed to correlate a number of parameters significant to lipid metabolism for both the whole organism as well as for isolated systems. The experimental model developed previously was used, with fat and protein as the dietary variables during the refeeding period. Of particular interest were correlations of overall indices of lipogenesis - accumulation of body fat, augmentation of fatty acids indicative of de novo fat synthesis, overall oxidation of ^{14}C-palmitate to $^{14}CO_2$ - with others related to lipid metabolism in adipose and/or hepatic tissue - NADPH generating enzymes and acetate-1-^{14}C incorporation into fatty acids.

REVIEW OF LITERATURE

Lipogenesis, the process by which the body takes chemical energy ingested in food in excess of the immediate energy requirements and converts it to a compact energy reservoir, has been studied extensively. The mechanism or mechanisms involved in the regulation of this process have not been determined. However, many facets of this process have been elucidated. Wakil (1961), Lynen (1961), and Vagelos (1964) were pioneers in clarifying the process of de novo biosynthesis of palmitic acid from acetyl-CoA by the extramitochondrial system. The mechanism is believed to consist of several reactions which can be summarized by the following two equations:

$$7 \text{ acetyl-CoA} + 7 \text{ ATP} + 7 \text{ CO}_2 \longrightarrow 7 \text{ malonyl-CoA} + 7 \text{ ATP} + 7 \text{ P}_i \quad (1)$$

$$7 \text{ malonyl-CoA} + \text{acetyl-CoA} + 14 \text{ NADPH} + 14 \text{ H}^+ \longrightarrow \text{palmitic acid} + 14 \text{ NADP}^+ + 7 \text{ CO}_2 + 8 \text{ CoA} + 6 \text{ H}_2\text{O} \quad (2)$$

Reaction 1 is catalyzed by acetyl-CoA carboxylase and is biotin dependent. At present it is thought to be the rate controlling step. A tightly bound complex of enzymes referred to collectively as fatty acid synthetase catalyze the second reaction.

Enzymatic pathways for the de novo synthesis of fatty acids are also present within the mitochondrion. The end product of this synthetic pathway is stearic acid. This system is much less active than the one located in the extramitochondrial

region.

Chain elongation and desaturation provide the body with a variety of fatty acids. The principal systems for these processes are found in the microsomes and thus are probably components of the endoplasmic reticulum of intact cells. The system for chain elongation reacts malonyl-CoA with a long-chain fatty acyl-CoA and through a series of reactions a fatty acyl-CoA two carbons longer than the initial acyl-CoA is formed. The reducing power of NADPH is used in this process. The system can elongate either saturated or unsaturated fatty acids and can continue for one or more cycles. The microsmal fraction of the liver also contains a system for desaturation of fatty acids. This system also utilizes NADPH.

The liver was long considered the major, if not the sole, site of lipogenesis in the mammalian organism. However, Leveille (1967a) has reported that the adipose tissue is the major site of synthesis in the rat. This worker suggests that if all body adipose tissue synthesizes fatty acids at the rate observed for epididymal adipose tissue, 89% of the fatty acids appear to be derived from adipose tissue, and if all but epididymal tissue is disregarded, about 50% of the fatty acids are synthesized by this tissue. That lipogenic activity of the adipose tissue is superior to that of the liver in rats was also reported by Hausberger and Milstein (1955).

Since the function of fatty acid biosynthesis is to store chemical energy from foodstuff, the rate of fatty acid bio-

synthesis is markedly influenced by the nutritional state of the animal. Measuring accumulation and composition of lipid, incorporation of labelled substrates into fatty acids, as well as activity of specific enzymes have been used to evaluate lipogenesis.

Effect of Diet on Lipogenesis

Liver

Changes in the fatty acid composition of tissue lipids due to dietary manipulation have often been correlated with lipogenesis. Noble (1967) and Stadler (1969) have extensively reviewed the effects of diet on fatty acid composition of hepatic and epididymal lipids. Therefore only a few of these studies and conclusions arrived from them will be reported by this author. Noble (1967) cited evidence that an increase in the four major saturated and monounsaturated fatty acids, palmitic, palmitoleic, stearic, and oleic occurs when lipogenesis is elevated in the absence of dietary fat. When fat is present in the diet the liver tissue fatty acid pattern reflects the incorporation of the dietary lipids, except for short chain fatty acids which are not incorporated. These short chain fatty acids cause an increase in the relative percentage of palmitic acid. That the changes observed in the fatty acid composition of total hepatic lipid are more pronounced in the nonphospholipid than the phospholipid fraction was evinced in numerous reports reviewed by Stadler (1969).

It became clear quite early that fasting for a few days drastically reduced the capacity of the liver to convert the ^{14}C of ^{14}C-glucose (Masoro et al., 1950), ^{14}C-fructose (Wyshak and Chaikoff, 1952) and acetate-1-^{14}C into fatty acids, and that a single administration of glucose, but not of protein or fat, completely restored hepatic lipogenesis of the fasted rat to normal.

Varying dietary fat was used as a tool to study lipogenesis by Hill et al. (1958). Increasing amounts of corn oil were incorporated into diets which contained 55% glucose and 22% casein. As the percentage of corn oil in the diet was increased from 0 to 10, the conversion of the added acetate-^{14}C to fatty acids by liver slices was decreased. No differences were observed between animals fed 15% corn oil and those fed 10% corn oil. With this 3 day feeding trial no changes were observed in the level of fatty acids in the liver. In a subsequent study these workers fed rats isocaloric diets containing 0-15% fat for 3 days after which they were injected with acetate-1-^{14}C. One hour later they were killed. Reduced recovery of ^{14}C in fatty acids with increasing amounts of dietary fat was again evident.

Bortz et al. (1963) reported experiments dealing with the nature and subcellular site of the block in lipogenesis by fat feeding. Animals were fed a fat-free diet containing 37% casein for 3 to 7 days. Before sacrifice, the animals received

by stomach tube, 2 ml of corn oil. Acetate-1-^{14}C, acetyl-1-^{14}C-CoA, and malonyl-1,3-^{14}C-CoA incorporation into fatty acids by liver slices or by a system composed of the supernatant and microsomal fractions was measured. Conversion of acetate carbon to fatty acids was depressed two hours after the administration of corn oil. Four hours after fat feeding the decrease in fatty acid synthesis was most pronounced. The depression in the incorporation of acetate into fatty acids was more pronounced in liver slices than in the system composed of the supernatant fraction plus microsomes. Fatty acid synthesis from acetyl-CoA was impaired in homogenate fractions prepared from rats receiving fat in the diet. The synthesis from malonyl-CoA was not affected. The authors conclude from these results that the block in lipogenesis due to dietary fat seems to be at the acetyl-CoA carboxylase step.

Fatty acid synthesis during fat-free refeeding of starved rats was studied by Allmann and coworkers (1965). Young male rats were fed a balanced stock diet for at least 3 days before an experiment was undertaken. Rats were then starved 48 hours and refed for 48 hours a balanced stock diet or a fat-free diet. Acetate-1-^{14}C, acetyl-1-^{14}C-CoA, and malonyl-2-^{14}C-CoA incorporation into fatty acids by the soluble supernatant fraction of the liver was measured. Incorporation of each of these substrates was decreased during starvation and elevated upon refeeding. Refeeding the balanced stock diet resulted in a 2-fold increase over control levels, while refeeding the fat-free

diet gave a 9-fold increase. The percentage of linoleic acid in the liver fatty acid fraction was inversely related to the level of fatty acid synthesis. Refeeding the fat-free diet resulted in a decrease in the relative concentrations of stearic and arachidonic acids. However, acetate incorporation into stearic acid as well as into palmitoleic and oleic acids by liver slices was enhanced during the fat-free refeeding period.

Noble (1967) used a less direct approach to study lipogenesis. Furthermore she used chronically starved adult rats in contrast to fasted refed young rats. During refeeding a low fat diet containing 4% protein was used. Hepatic total lipids increased markedly during the first 24 hours. Palmitoleic and oleic acids accumulated. These changes were confirmed by Stadler (1969). Investigations by this worker indicated that the increase in percentage of palmitoleic and oleic acids was reflected to a greater extent in the non-phospholipid fraction of hepatic lipids than in the phospholipid fraction.

Recently Kimura and Ashida (1969a) studied the influence of dietary carbohydrate, fat and protein on lipogenesis in young rats. These workers observed a rapid drop in lipogenesis in the liver with an increase in corn oil content in starch-corn oil diets, while lipogenesis decreased gradually with an increment in casein content of starch-casein diets. In a subsequent study (Kimura, 1969b) animals were trained to

consume a 25% casein diet for only 2 hours a day. After the training period rats were given 5 g of starch, 5 g of casein, or 2 g of corn oil with 3 g of cellulose powder. At the end of 2 hours of feeding, the animals were injected intraperitoneally with acetate-1-^{14}C and placed in a metabolic chamber for 1 hour. Respiratory $^{14}CO_2$ was lowered when starch was fed, but no differences could be detected between animals fed casein or corn oil. In accordance with the respiration data incorporation of ^{14}C into liver lipids was greatly elevated in animals receiving starch and only slightly raised when casein was fed.

Tepperman et al. (1968) studied the effects of dietary protein on lipogenesis. After a 48 hour fast rats were given low fat diets containing zero protein or 33% protein. Lipogenesis by liver slices was just as high when the zero protein diet was refed as it was on the 33% protein diet. These authors suggest that the enzymes of lipogenesis appear to adapt well without a continuing exogenous source of amino acids. Hill et al. (1958) also found that hepatic lipogenesis was not effected by varying dietary protein from 15 to 37%.

In general hepatic lipogenesis is decreased during fasting and is elevated over normal values upon refeeding a high carbohydrate diet. This elevation upon refeeding is depressed greatly by substitution of fat in the diet and to a lesser extent by substitution of protein for carbohydrate in the diet. These changes were observed also if fat or protein were fed for

a long period of time to young rats without a previous period of fasting.

Adipose tissue

The epididymal fat pad has been used extensively to indicate changes in lipogenesis of adipose tissue. Unless otherwise specified references to adipose tissue in the reports to be presented means epididymal tissue. The adipose tissue seems to reflect the same changes in lipogenesis due to dietary manipulation as the liver. In some instances a longer feeding period is needed for this tissue to reflect changes due to the diet. Noble (1967) has cited reports that show that the percentage of palmitic acid is quite constant in adipose tissue of rats fed low fat diets. Palmitic acid is usually 25 to 30% of the total fatty acids. Further evidence is cited that high carbohydrate (94% sucrose) or high protein (96% casein) diets without added fat produce similar results.

An elevation of the relative percentage of palmitoleic acid when low fat diets are fed was evinced in numerous reports quoted in Noble's review. Palmitoleic acid usually comprises 3 to 5% of adipose lipids from rats fed stock diets, and 10 to 20% of adipose lipids from rats fed low fat diets. Another characteristic of adipose fat reported in many of the studies in the review is the low concentration of the polyenoic acids when these are absent from the diet.

Another portion of Noble's review deals with the effects of diets containing fat on the fatty acid composition of adipose lipids. Studies indicate that body fat usually consist of fatty acids with chain lengths of 16 and 18 carbon atoms. This can be modified by dietary fat containing medium chain fatty acids which are also deposited. Incorporation of 10 to 14 carbon fatty acids into epidiymal lipid was noted by Kopec (1969). These medium chain fatty acids comprised 14% of the fatty acids with refeeding diets containing butter oil. Feeding diets containing short chain fatty acids usually results in an increase in the level of palmitic acid.

Dietary effects on lipogenesis in adipose tissue was studied by Hausberger and Milstein (1955). These workers fed special diets to rats weighing approximately 300 g for at least 14 days. Glucose-1-^{14}C incorporation into tissue slices was used as a measure of lipogenesis. Lipogenesis was abolished during prolonged fasting, which lasted for 8 days. The same results were obtained with a diet containing 60% fat, 1.2% carbohydrate, and 26% protein. A diet composed of equal amounts of carbohydrate and fat with 15% protein induced a slight stimulation of fat synthesis. A fat-free, high carbohydrate diet, however, increased adipose tissue lipogenesis to the greatest extent of any regime tested.

Further information on relative intensity of lipogenesis with respect to time was obtained by Allmann and coworkers (1965). Lipogenesis was measured by pyruvate-2-^{14}C incor-

poration into fatty acids by intact epidiymal fat pads. Fatty acid synthesis decreased during a 48 hour fast, but began to be restored as early as 6 hours after refeeding and attained a level 3 times the level found in controls at the end of 24 hours. At the end of the 48 hour refeeding period the fatty acid composition of adipose lipid had changed, with marked increases in palmitoleic and oleic acids, and decreases in stearic and linoleic acids. Increased incorporation of acetate-1-^{14}C into the monoenes by adipose tissue from rats fed a fat-free diet was also reported by Gellhorn et al. (1962).

Similar changes have been seen in chronically malnourished adult rats. These animals differ from the fasted animals discussed earlier in that they are mature and have undergone extensive depletion of expendable lipid and protein reserves. Noble (1967) found increases in percentages of palmitoleic and oleic acids in adipose lipids. These changes were apparent after only 3 days of refeeding (Kopec, 1969), but larger percentages of these fatty acids were found after 10 days of refeeding. Palmitic acid was also elevated in epididymal lipid after this time (Stadler, 1969).

The influence of dietary fat and protein on fatty acid synthesis of adipose tissue was studied by Leveille (1967b, 1967c). When young rats were fed a diet containing 80% of calories as carbohydrate, pyruvate-2-^{14}C incorporation into fatty acids by adipose tissue was approximately 40 times greater than that by fat pads from animals given fat as 80%

of the calories. In a subsequent study diets containing 10, 20, or 30% fat were fed. The fat was added at the expense of glucose. A significant depression in the incorporation of acetate-1-^{14}C and glucose-U-^{14}C into fatty acids was observed when the level of dietary fat was increased from 10 to 20%. The further increase of dietary fat to 30% of the diet had no significant effect on fatty acid synthesis from either substrate. Utilization *in vitro* of glucose-U-^{14}C and L-leucine-U-^{14}C by adipose tissue of rats fed diets containing 9, 18, or 36% protein was also measured. Fatty acid synthesis from both glucose and leucine was depressed markedly by increasing dietary protein. This author suggests that this depression could be due to decreased carbohydrate rather than an effect of protein per se, since protein was added to the diet at the expense of glucose.

Changes in lipogenesis described for epidiymal fat pads have been observed in other adipose tissue. Lipogenesis, as measured by the *in vivo* incorporation of acetate-1-^{14}C into carcass lipids was elevated after rats received 5 g of starch (Kimura, 1969). Feeding 5 g of casein or 2 g of corn oil did not affect the rate of lipogenesis compared to control values. A decrease in incorporation of acetate-1-^{14}C into lipid by skin of starved rats has recently been reported by Ziboh and Hsia (1969).

Effect of Diet on NADP-Linked Enzymes

Cofactors available for use in the synthesis of fatty acids were at one time considered to be the prime regulators of fatty acid biosynthesis. The major cofactor investigated has been NADPH which is required for the de novo synthesis of fatty acids and also for the elongation and desaturation of long chain fatty acids. Langdon (1955) recognized early the importance of the two NADPH-generating enzymes of the hexose monophosphate shunt, glucose-6-phosphate dehydrogenase (G-6-PD, EC1.1.1.49) and 6-phosphogluconate dehydrogenase (6-P-GD, EC 1.1.1.44). Subsequently these two enzymes have received considerable attention and were considered to produce all the reducing power needed for fatty acid synthesis. Elegant experiments by Flatt and Ball (1964) and Rognstad and Katz (1966) demonstrated that only about 50% of the NADPH needed for high rates of lipogenesis in rat adipose tissue could be supplied by the hexose monophosphate shunt dehydrogenases (HMPD). This led to a search for other pathways that would generate NADPH.

Experiments of Young *et al.* (1964) and Wise and Ball (1964) suggested that oxaloacetate derived from citrate cleavage could be converted to malate then to pyruvate utilizing malic dehydrogenase and malic enzyme, and thus transfer hydrogen from NADH to $NADP^+$. Tepperman and Tepperman (1964) had noted an increase in NADP-malate dehydrogenase (MDH, EC 1.1.1.40) in states of heightened lipogenesis, but did not recognize its significance. After finding evidence that

pyruvate carboxylase was present in both cytosol and mitochondria of adipose tissue, Ballard and Hanson (1967) proposed a "transhydrogenation cycle", which would consist of the following 3 reactions:

$$\text{oxaloacetate} + \text{NADH} \longrightarrow \text{malate} + \text{NAD}^+ \qquad (1)$$

$$\text{malate} + \text{NADP}^+ \longrightarrow \text{pyruvate} + \text{NADPH} + \text{CO}_2 \qquad (2)$$

$$\text{pyruvate} + \text{CO}_2 + \text{ATP} \longrightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i \qquad (3)$$

These workers propose that this cycle could operate in conjunction with the citrate cleavage enzyme with reaction 3 occurring intramitochondrially to replenish the oxaloacetate, or that it might work separately with reaction 3 occurring in the cytoplasm for the generation of extramitochondrial NADPH. Henning et al. (1966) have also reported a soluble and mitochondrial pyruvate carboxylase in rat liver. In their studies changes in dietary conditions which favor gluconeogenesis in liver were accompanied by an increase in pyruvate carboxylase activity in both cell fractions. These findings could not be confirmed by Krebs (1965) or by Shrago and Lardy (1966). Ballard and Hanson (1967) noted a decrease in the activity of the soluble enzyme in adipose tissue of rats that were fasted and then fed a high fat diet, a process which greatly enhances hepatic gluconeogenesis, and suggests a different metabolic function of pyruvate carboxylase in liver from that in adipose tissue. However, this does not exclude the possibility that NADPH for hepatic lipogenesis is furnished by NADP-malate dehydrogenase.

Isocitrate dehydrogenase (IDH, EC 1.1.1.42) has been suggested as a further possible source of reducing power. Leveille (1970) considers this unlikely since it would probably operate in the opposite direction to carboxylate a-ketoglutarate to supply citrate for the citrate cleavage enzyme. However, Greville (1969) suggests that the dehydrogenase may serve as a source for part or all of the large amount of NADPH required for fatty acid synthesis if the mitochondrion specifically exports citrate or isocitrate. Recent work by Hanson (1970) demonstrating the efflux rates of citrate from the mitochondria lends support to the theory regarding the production of NADPH by isocitrate dehydrogenase for fatty acid synthesis.

Many variations in dietary regimens have been used to elucidate the relationship between activity of the enzyme systems that produce NADPH and lipogenesis. This has been one of the most active areas of lipid research and has led to what Leveille (1970) has termed the "literature explosion". This review will cover a limited number of studies on rats illustrating the general findings in the field.

Both hepatic and adipose sites have been studied. Adipose tissue usually had higher HMPD and MDH activity than did liver, whereas IDH activity was higher in liver than adipose tissue (Pande et al., 1964; Young et al., 1964).

Liver

Hexose monophosphate shunt dehydrogenases Glock and McLean (1955a) compared hexose monophosphate shunt dehydrogenase activity of young male rats fed ad libitum to those receiving 5, 10, or 15 g of a complete diet. Animals were fed for 8 days or starved for 48 hours prior to autopsy. The dehydrogenase activities were higher in the animals fed ad libitum than in those restricted in food intakes. In another study (1955b) these workers found shunt activity was less after 48 than after 24 hours of starvation. Since this time the technique of fasting animals followed by refeeding has been used by many workers to determine factors affecting the activity of the shunt enzymes.

The response of HMPD to refeeding a high carbohydrate diet seems to occur very rapidly, since Tepperman and Tepperman (1958) noted increased activity after only 3 hours. The HMPD activity reached a maximum on the third day of refeeding and was still elevated after 5 days. These workers also reported a net accumulation of fat after 24 to 48 hours of refeeding. Substitution of fat for carbohydrate and protein in the diet decreased HMPD activity and lipogenesis as measured by acetate-1-^{14}C incoporation into fatty acids (Tepperman and Tepperman, 1963). The depression of lipogenesis was immediate, but shunt activity did not decrease for 2 or 3 days. The authors suggested several reasons for the lag between depression of fatty acid synthesis and cessation of the overshoot in HMPD activ-

ity. Either there was no association between lipogenesis and HMPD activity or considerable searching for the pentose pathway "shut off" signal, or the basic pattern permitted "overshooting" of activity. Adaptive hyperlipogenesis, a state of increased fatty acid synthesis accompanying refeeding with high carbohydrate, may also occur with only small increases in HMPD activity (Tepperman et al., 1968). The uncoupling of adaptive lipogenesis from increased HMPD activity led these workers to pose a very interesting question which remains unanswered. If high levels of hepatic lipogenesis can be attained with only modest increases in HMPD activity, what is the physiologic significance of the very large increases in HMPD activity that occur on refeeding?

Refeeding chronically malnourished rats elicits responses in hepatic HMPD activity similar to those in refeeding previously fasted rats. Thorp (1966) in this laboratory subjected adult male rats to successive periods of protein depletion, protein depletion plus food restriction, and realimentation with diets containing 4% lactalbumin protein for either 3 or 10 day. Corn starch or corn starch plus 20% corn oil was fed as the source of food energy during refeeding. After three days of refeeding with only carbohydrate as the energy source, HMPD activity was increased to 8 times the control value and this level was maintained up to the tenth day of realimentation. In contrast, realimentation with the 20% diet provided only a two fold increase in activity after 3 days; after 10

days the HMPD activity had returned to the control level.

Depression of HMPD activity following the inclusion of fat in the diet has also been found in rats not subjected to previous periods of fasting or starvation (Vaughan and Winders, 1964; Baldwin et al., 1966; and Konishi, 1966). Substitution of fat for either casein or sucrose, even at the 15% level markedly reduced the HMPD activity and further substitution continued this trend (Vaughan and Winders, 1964). Even the type of carbohydrate used in refeeding seems to have an influence since depression of HMPD activity was seen when dextrin or corn starch was substituted for glucose. Vaughan and Winders (1964) proposed the following mechanism: fat or complex carbohydrates in the diet suppress HMPD activity by spreading out glucose uptake, so that the liver does not receive large intermittent loads. This theory was substantiated by the finding that fat, offered concurrently with carbohydrate, depressed activity of both enzymes, but had little effect when given on alternate days. Another theory regarding the mechanism by which dietary fat suppresses HMPD activity was proposed by Baldwin et al. (1966). These workers suggest that enzymatic changes were related to the caloric contributions rather than the amounts of carbohydrate per se in the diets. This theory was based on the observation that HMPD activity decreased logarithmically as the ratio of calories from carbohydrate to calories from carbohydrate plus fat in the diet decreased.

Dietary protein seems to be necessary for "overshoot" in HMPD activity which occurs upon refeeding. Niemeyer et al. (1962) failed to obtain increases in HMPD activity over fasting values with a N-free refeeding diet. In studies where stimulation of HMPD has been found with N-free diets, the stimulatory effect was quite small (Vaughan and Winders, 1964; Tepperman et al., 1968). Stimulation of HMPD activity is also hindered when dietary protein is low. Potter and Ono (1961) obtained no stimulation with a 2% casein refeeding diet. McDonald and Johnson (1965) found a 3 fold increase in shunt activity with a 4% protein diet after starvation, but a 14 fold increase was obtained with an 18% protein diet.

The induction of HMPD is thought to be dependent on both dietary protein and carbohydrate, Potter and Ono (1961) found no increase in HMPD activity when diets containing 91% casein were refed after a 3 day fast. In contrast a 3 fold increase in HMPD activity after refeeding a 91% protein diet was reported by Vaughan and Winders (1964). These workers suggested that on high protein diets large amounts of amino acid residues are diverted toward glucose-6-phosphate and thence through the HMP shunt system, instead of flowing immediately through the TCA cycle. Reasoning that alanine would be one of amino acids most involved, Vaughan and Winders (1964) supplemented the high protein diet with 17% alanine. HMPD activity was significantly increased over that seen with the unsupplemented diet. Szepesi and Freedland (1968) also reported that high protein

induced G-6-PD after prefeeding with a protein free diet. In contrast high protein diets depressed G-6-PD activity when fed after a diet containing adequate protein.

The effects of dietary protein on shunt activity in chronically starved rats has been studied by Lee (1967) in this laboratory. Animals were refed diets containing 0, 5.1, 16.5, or 49.5% of calories as protein for 3 days. Response of HMPD activity in these animals appeared to be similar to that reported by others for fasted refed rats. A small stimulatory effect was produced with the N-free diet. The response of HMPD activity to the low and high protein diets was similar, levels exceeded the control value by approximately 5 times. However, maximum activity of HMPD was seen with the intermediate level of protein. Substitution of 20% fat for carbohydrate in the 16.5% and 49.5% protein diets decreased the activity of HMPD. Activity was elevated over control values only when protein supplied 16.5% of the calories.

NADP-malate dehydrogenase (MDH, EC 1.1.1.40) The effects of dietary manipulations on hepatic NADP-malate dehydrogenase have been studied in recent years. Several trivial names have been used for this enzyme, but malic enzyme seems to be favored. Effects of starvation and dietary fat on MDH activity are similar to those on HMPD. Activity of MDH was reduced during starvation (Freedland, 1967). Depression of MDH activity when fat is substituted for carbohydrate in the diet has also been reported (Vaughan and Winders, 1964;

Tepperman and Tepperman, 1964; Baldwin et al., 1966; Leveille, 1967a). Substitution of complex carbohydrates, dextrin and corn starch for glucose in the diet appears to lower MDH activity (Vaughan and Winders, 1964).

NADP-malate dehydrogenase activity does not exhibit as great a dependency on dietary protein as does HMPD (Vaughan and Winders, 1964; Szepesi and Freedland, 1968). An excellent example of this is seen in experiments by Tepperman et al. (1968). Animals were fasted for 48 hours and then refed a diet containing either zero or 33% protein. A 17-fold increase in malic enzyme occurred in animals on the 33% protein diet, while the low protein diet produced a 4-fold increase. Incorporation of acetate into fatty acids by liver slices was elevated to the same extent on both diets, indicating that lipogenesis was not affected by protein. These workers calculated the μmoles of TPN/min per mg N reduced by both MDH and HMPD. The homogenates prepared from the livers of animals refed 33% protein containing diets were capable of reducing 130 μmoles of TPN/min per mg N, while the corresponding figure for zero protein diets was 13 μmoles. Based on the fact that there was no statistically significant difference in acetate incorporation into fatty acids these authors concluded that 0.9 of the measurable TPNH generating activity of the homogenates is questionably relevant to the acetate to fatty acid conversion performance of liver slices. In another experiment a comparison was made between feeding a 89% protein, 11% fat

diet or a 89% sucrose, 11% fat diet. Both diets caused an elevation in activity of MDH but the response was greater with sucrose than with protein.

Using dietary manipulations as a tool for studying the mechanisms of response of NADP-malate dehydrogenase has made it possible to draw some conclusions. Fat inclusion in the diet has been found to decrease the activity of this enzyme as well as lipogenesis. NADP-malate dehydrogenase activity can be induced without a dietary supply of protein as can the enzymes of lipogenesis. However, greater induction of this enzyme is possible if adequate quantities of protein are included in the diet, while high quantities of protein suppress this induction.

NADP-isocitrate dehydrogenase (IDH, EC 1.1.1.42)

Hepatic NADP-isocitrate dehydrogenase is always high in comparison to that in other tissues. Here as with the other two enzyme systems fasting seems to lower the activity (Pande et al., 1964). But in contrast to HMPD and MDH the activity of IDH does not seem to increase upon refeeding after a fast (Pande, 1964; Young et al., 1964). Small stimulatory effects have been reported after refeeding starved, adult male rats. McDonald and Johnson (1965) starved adult male rats until their body weight was reduced by 20%. After starvation animals were refed diets containing 4 or 18% protein for varying periods from 12 to 96 hours. IDH activity was decreased during starvation. The activity tended to be lower when protein was fed at

the 4% level than when fed at 18%. Mean values and standard deviations for isocitrate dehydrogenase activity were 103 ±33 in animals fed 4% protein compared to 164±31 with 18% protein. Isocitrate dehydrogenase did not start to increase until after 48 hours of refeeding.

From the papers discussed above it appears that the activity of hepatic isocitrate dehydrogenase is not greatly influenced by periods of fasting and refeeding. In this respect this enzyme behaves differently from the hexose monophosphate shunt dehydrogenases and MDH for IDH activity is high in livers from well fed animals and tends to decrease during fasting and starvation, but no overshoot in activity during refeeding has been observed. However, usually short refeeding periods have been used and McDonald and Johnson reported a lag of 48 hours before IDH activity began to increase upon refeeding. The data of McDonald and Johnson (1965) suggest a possible stimulatory effect of dietary protein in refeeding diets after chronic starvation.

Adipose tissue

Hexose monophosphate shunt dehydrogenases In the epididymal tissue the hexose monophosphate shunt dehydrogenases respond to dietary manipulations in much the same manner as do the hepatic dehydrogenase. However, the activity seems to be higher in epididymal tissue than in liver (Pande, 1964; Young et al., 1964). Glucose-6-phosphate dehydrogenase activity

decreased during fasting and displayed the typical overshoot in activity upon refeeding (Young et al., 1964). Depression of activity by dietary fat has also been reported. Konishi (1966) fed weanling rats diets containing 10 or 45% fat and 25% casein for 12 weeks. Fat was added to the diet at the expense of sucrose. Animals receiving diets containing 45% fat had only 16% of the G-6-PD activity seen in animals fed 10% fat. Depression of HMPD activity by dietary fat has been confirmed by Leveille (1967b, 1967c) and Fabry et al. (1970).

The relationship of dietary protein to activity of HMPD in epididymal fat pads has been studied less extensively than in hepatic tissue. But one pattern seems to emerge: increasing dietary protein beyond adequate levels of intake seems to depress HMPD activity, as demonstrated by the study reported by Konishi (1966). Weanling rats were fed for 12 weeks a diet containing 10% fat and 25 or 55% casein. Fat pads from animals fed diets containing 25% protein had 4 times the G-6-PD activity as did fat pads from animals fed the 55% protein diet.

NADP-malate dehydrogenase Activity of malic enzyme in epididymal tissue appears to be higher than in hepatic tissue (Pande, 1964; Young et al., 1964). Starvation, and the inclusion of fat or protein in the diet have been reported to depress MDH activity.

Young et al. (1964) were among the first workers to recognize the significance of malic enzyme to lipogenesis. Rats

were fasted for periods varying from 24 to 96 hours, then refed a standard chow diet. Malic enzyme activity decreased during a 96 hour fast and increased during 96 hours of refeeding to values 6 times those of nonfasted controls. Fasting for 96 hours led to a greater refeeding response of malic enzyme to refeeding than fasting for 48 hours and this response was maintained over a longer period of time. These authors suggest that after a 96 hour fast a higher rate of lipogenesis would be required to restore body fat stores to normal than after a 48 hour fast.

Leveille (1967b) studied the effect of fat on MDH activity of rat epididymal fat pads. When rats were fed diets containing 80% of calories as glucose, MDH activity was 6 times higher than the value found when fat was substituted for glucose in the diet. In a subsequent study (Leveille, 1967c) increasing dietary fat from 10 to 20% of the diet decreased MDH activity, but an additional increase to 30% had no effect.

The depression in MDH activity observed upon fat feeding was confirmed by the study of Fabry et al. (1970). Animals were fasted for 72 hours and refed either a high carbohydrate or a high fat diet. Malic enzyme activity in adipose tissue from rats fed the high carbohydrate diet was 400% of the activity found in rats fed the high fat diet.

Protein was used as a dietary variable in a study conducted by Leveille (1967b). Diets containing 9, 18, or 36%

protein were fed young rats for 3 weeks. The rest of the energy source was glucose. The levels of MDH activity in epididymal fat pads from animals fed 9 or 18% protein were not significantly different. When protein was increased to 36% of the diet the level of activity of MDH was depressed to about 1/2 of the values observed in the other groups.

NADP-isocitrate dehydrogenase In contrast to HMPD and MDH activity, isocitrate dehydrogenase is low in adipose tissue and does not seem to be induced by refeeding fasting rats. Young et al. (1964) reported a relatively low level of IDH in adipose tissue which did not adapt to increased lipogenesis. These workers suggest that this may be an indication that the enzyme is not directly related to fatty acid synthesis as a NADPH-generating system. The low value of epididymal IDH when compared to hepatic IDH was also reported by Pande et al. (1964) They report isocitrate dehydrogenase in adipose tissue being only 13% of the level observed in the liver.

METHODS AND PROCEDURES

Selection and Treatment of Animals

Experimental plan

Pre-experimental Male adult rats were taken from the colony at Iowa State University, which was established in 1962, from breeding stock of the Wistar strain.[1] They were weaned at the age of 28 days and thereafter raised on the modified Steenbock XVII ration (Table 1). The diet was supplemented weekly with 15 gm of lean ground beef, 20 g of carrots, 10 g of cabbage, and 165 mcg of vitamin A acetate. The stock diet contained 7.4% fat. Analysis of the fatty acid composition of the stock diet and of corn oil have been reported previously from this laboratory (Stadler, 1969) (Table 2). Animals 4-1/2 to 6 months of age and weighing approximately 500 g were taken for the experiment. Animals were fasted over night and either autopsied, Group I, or placed on dietary treatment.

Period 1 Animals were individually housed in one-half inch mesh, suspended wire cages in a laboratory maintained at $24 \pm 1^{\circ}C$ with a minimum relative humidity of 40%. Food intake and body weights were recorded every two days. The experimental dietary regimen consisted of two periods; depletion and rehabilitation. In the first phase protein stores were depleted by feeding a protein-free diet ad libitum. This was

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[1] Simonsen Laboratories, White Bear Lake, Minnesota.

Table 1. Stock ration for male rats, Steenbock XVII

Dietary component	Percent
Corn meal[a]	48.3
Linseed meal[b]	13.8
Dry skim milk[c]	10.2
Wheat germ[d]	8.6
Yeast, Brewer's USP[a]	8.2
Casein, crude B3F[e]	4.3
Cottonseed oil[f]	3.6
Alfalfa meal[g]	1.7
NaCl[h]	0.4
$CaCO_3$[i]	0.4
Yeast (irradiated)[j]	0.4

[a]General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Froning and Deppe Elevator, Ames, Iowa.

[c]Des Moines Cooperative Dairy, Des Moines, Iowa.

[d]General Mills, Inc., Chicago, Illinois.

[e]The Borden Company, Chemical Division, New York.

[f]Wesson Oil, Wesson Sales Company, Fullerton, California.

[g]National Alfalfa, Lexington, Nebraska.

[h]Analytical reagent, Mallinckrodt Chemical Works, St. Louis, Missouri.

[i]Matheson Coleman and Bell Divison of the Matheson Company, Inc., Norwood, Ohio.

[j]Brewer's yeast irradiated in this laboratory.

Table 2. Fatty acid composition of stock ration, Steenbock XVII, and of corn oil.

	%Fatty acids						
	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Stock diet	0.6	17.8	2.1	2.7	21.7	49.4	5.7
Corn oil	-	11.0	-	2.4	27.8	57.1	1.8

followed by severe caloric restriction induced by feeding the protein free diet at approximately one-fifth of the ad libitum intake, divided into two daily portions. Caloric restriction began when the animal's body weight dropped below 400 g if this occurred after 25 days of protein depletion. Animals whose body weight was between 375 and 400 g on the 25th day of depletion were placed on caloric restriction at that time. Animals remained on the restricted diet until their body weight was reduced to at least 300 g. Animals sacrificed at that time were designated group II.

Period 2 This was a 10 day period of nutritional rehabilitation with ad libitum consumption of a diet containing 1.08 (P), 3.23 (3P), and 9.68 (9P) g of protein per 100 kilocalories from lactalbumin. The balance of calories was supplied by corn starch or corn starch plus 20% corn oil. Ratio of calories to mineral mix and non-nutritive fiber were equalized for all diets. Components of the experimental diets are listed in Table 3. These six groups were designated III-

Table 3. Composition of experimental diets

	II OF-OP	III OF-P	IV OF-3P	V OF-9P	VI 20F-P	VII 20F-3P	VIII 20F-9P
				% weight			
Corn starch[a]	93.0	87.9	77.7	47.2	65.4	52.5	14.3
Corn oil[b]	-	-	-	-	20.0	20.0	20.0
Lactalbumin[c]	-	5.1	15.3	45.8	6.4	19.3	57.5
Hawk and Oser salt[d]	4.0	4.0	4.0	4.0	5.0	5.0	5.0
Non-nutritive fiber[d]	2.8	2.8	2.8	2.8	3.0	3.0	3.0
NaCl[e]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
				% kilocalories			
Corn starch	100.0	94.5	83.5	50.8	56.0	44.9	12.2
Corn oil	-	-	-	-	38.5	38.5	38.5
Lactalbumin protein	-	4.3	13.0	38.7	4.3	13.0	38.7

[a]Argo, Best Foods Division Corn Products Company, New York, New York.

[b]Mazola, Best Foods Division Corn Products Company, New York, New York.

[c]78.6% protein, Nutritional Biochemical Corporation, Cleveland, Ohio.

[d]General Biochemicals, Inc., Chagrin Falls, Ohio.

[e]Matheson Coleman and Bell Division of the Matheson Company, Inc., Norwood, Ohio.

VIII. The calculated physiological fuel value was 3.72 kilocalories per g for diets without fat and 4.67 kilocalories per g for the diets with fat. The experimental diets were supplemented daily with 500 mg of a mixture containing crystalline, water soluble vitamins (Table 4) plus 50 mg cod liver oil[1] and 0.75 mg dl-alpha tocopherol[2] in 50 mg of cottonseed oil.[3] The cottonseed oil provided 25 mg linoleic acid (Watt and Merrill, 1963). Animals were permitted ad libitum intake of distilled water.

Experimental groups The length of time each animal remained on the dietary regimen of period 1 varied from 36 to 75 days, according to his individual rate of weight loss. Animals were assigned to groups III-VIII at the end of period 1 so that both initial body weight and the length of period 1 were as uniform as possible throughout the experimental groups. In Experiment 1 all groups contained 10 rats. In Experiment 2 each dietary treatment group contained 15 rats. Analysis of variance showed there were no significant differences between groups in body weights, food intake, or length of days in period 1 (Tables 5 and 6).

Autopsy In Experiment 1 animals were fasted for the last 12±1 hours of the last day of the refeeding period. They

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[1]Squibb & Sons, New York, New York, 1700 USP vitamin A, 170 USP vitamin D per gm.

[2]General Biochemicals, Inc., Chagrin Falls, Ohio.

[3]Wesson Sales Company, Fullerton, California.

Table 4. Composition of water-soluble vitamin mixture

Vitamin[a]	Allowance per day	
Thiamine HCl	40	mcg
Riboflavin	60	mcg
Pyridoxine HCl	40	mcg
Ca-pantothenate	100	mcg
Nicotinic acid	500	mcg
Folic acid	8	mcg
Biotin[b]	1	mcg
Vitamin B_{12}[c]	0.75	mcg
Ascorbic acid	1	mg
Choline chloride	5	mg
Inositol	10	mg
P-amino-benzonic acid	10	mg
Dextrin to make	500	mg

[a]General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Biotin was mixed with dextrin so that 100 mg of the mixture contained 1 mg of biotin.

[c]Vitamin B_{12} in mannitol furnished 0.1 mg of vitamin B_{12} per 100 mg of mixture.

Table 5. Experiment 1-body weights and length of depletion phases, period 1

Group		Body weight Initial gm	Body weight Final gm	Days Phase 1	Days Phase 2	Food intake[a] Phase 1 gm/day
I	CS	488±5	-	-	-	-
II	CR	490±6	292±3	34±2	21±0.8	16.0±.8
III	OF-P	483±3	293±1	29±2	20±1.5	15.3±.7
IV	OF-3P	482±4	294±1	30±2	22±1.2	16.2±.8
V	OF-9P	482±4	295±1	30±2	20±1.0	16.4±.7
VI	20F-P	483±5	293±1	33±2	20±0.8	17.2±.6
VII	20F-3P	483±5	294±1	31±2	20±0.9	16.2±.8
VIII	20F-9P	481±4	295±1	29±2	21±1.3	15.9±.8

[a]Intake for phase 2 was 4 gm/day in both experiments.

Table 6. Experiment 2-body weights and length of depletion phases, period 1

Group		Body weight Initial gm	Body weight Final gm	Days Phase 1	Days Phase 2	Food intake Phase 1 gm/day
I	CS	482±2	-	-	-	-
II	CR	491±2	294±3	31±2	20±1	16.6±.8
III	OF-P	487±2	295±1	31±2	20±1	16.5±.4
IV	OF-3P	485±1	294±1	31±1	18±1	17.0±.6
V	OF-9P	488±1	293±1	31±1	20±1	16.9±.7
VI	20F-P	487±1	294±2	30±1	19±1	16.7±.4
VII	20F-3P	487±2	294±1	30±1	19±1	18.5±.5
VIII	20F-9P	484±1	292±2	31±1	20±1	17.5±.5

were then give 1 g of their experimental diet and 1 hour later injected with 10 μc palmitate-1-^{14}C, then placed in an oxidation chamber for 4 hours. As soon as the rats were taken from the chamber they were anesthetized by intraperitoneal injection of 125 mg sodium pentobarbital.[1] Animals in Experiment 2 were fasted 12$\pm$1 hours prior to sacrifice by stunning and partial decapitation. Livers and epididymal fat pads were removed and placed on moist filter paper over ice, where extraneous matter was trimmed off. Tissues were weighed and samples representative of the tissue were weighed for individual analyses. Remaining tissue was stored under nitrogen at -20°C for later analysis of lipid and fatty acid content.

Chemical Analyses

Lipids and fatty acids

Total lipid extraction The method of Folch, Lees, and Stanley (1957) as modified by Stadler (1969) was used to extract total lipid from liver and epididymal tissue. Final volume was 5 ml.

Gravimetric determinations A one ml aliquot of lipid extract was pipetted into an aluminum foil weighing cup previously brought to constant weight. The chloroform was evaporated with nitrogen. The cup was then heated at 100°C for 20 minutes, placed in a desiccator and weighed, when cooled.

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[1]Nembutal Sodium, Abbott Laboratories, North Chicago, Ill.

Separation of hepatic lipid fractions Borgstrom's (1952) procedure as modified by Stadler (1969) for silicic acid chromatography was used to separate liver lipid into non-phospholipid and phospholipid fractions. Completeness of separation was confirmed by thin-layer chromatography.

Esterification A modification of the procedure developed by Stoffel and coworkers (1959) was used to methylate fatty acids in total lipid extracted from epididymal fat pads and in lipid fractions obtained from liver extracts. The esterification agent used was 2% sulfuric acid in methanol. Esters were extracted with hexane.

Gas-liquid chromatography Methyl esters of fatty acids were analyzed by a Varian Aerograph,[1] flame detector, model 204B Chromatograph. Conditions for the analysis were as follows: column-10' x 1/8" stainless steel, packed with 3% ethylene glycol succinate on Chrom G 100/120 mesh HP; temperature-180°C; carrier gas flow-30 ml/min of helium.

Identification of fatty acids Peaks were identified by comparison with commerical standards obtained from Hormel Institute[2] and Applied Science Laboratories.[3] Methyl esters

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[1]Varian Aerograph, 2700 Mitchell Drive, Walnut Creek, California.

[2]University of Minnesota, Austin, Minnesota.

[3]Applied Science Laboratories, State College, Penn.

of arachidonic and docosahexaenoic acids were also obtained and incorporated into standards.

Measurement of fatty acids An Altek Peakometer[1] was used for obtaining areas under the peaks. Correction factors were obtained from the standards by dividing the known weight percents by the area percents obtained from peak area calculation. Corrected peak areas in the samples were then divided by the total area to obtain the relative percent of each component fatty acid. Peak areas and all subsequent calculations were done by computer programming.[2]

Determination of radioactivity of lipid fractions Lipids and fatty acids were plated at infinite thinness. During plating planchets turned continuously on an automatic planchet turner to allow for even distribution of sample. A lucite planchet holder was used to store plated samples until time of counting. The activity of the samples was monitored with a gas flow detector[3] attached to a low background planchet sample changer.[4]

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[1]Altek Associates, Arlington Heights, Illinois.

[2]Iowa State University Computer Programming Service Center.

[3]Model D47, Amersham/Searle, Des Plaines, Illinois.

[4]Model 1043, Amersham/Searle, Des Plaines, Illinois.

Preparation of palmitate-1-^{14}C The palmitate-1-^{14}C[1] in benzene[2] was divided into individual vials containing 10 μc of ^{14}C and 1 mg of carrier palmitate[3] and stored under nitrogen at -20°C. Just prior to use the benzene was evaporated under a stream of N_2 and the palmitate dissolved in 0.5 ml of 5% Tween 20[4] with heating in a hot water bath (80-85°C). The vial was rinsed with 1 ml of distilled water.

Measurement of $^{14}CO_2$ The animals were fasted for the last 12 hours of the refeeding period, after which they were given one gram of their respective diet. One hour later they were injected intraperitoneally with the 10 μc of the isotope and the rinsings and were immediately placed into a glass respiration chamber. The apparatus used for measurement was a modification of that used by Tolbert (1956). A tank of compressed air forced the expired air out of the animal chamber at a flow rate of approximately 250 ml per minute. The air was dried with drierite before entering the ionization chamber and the $^{14}CO_2$ trapped in ascarite before elimination of the air through a wet test meter into the atmosphere. The radioactivity was measured by the "high resistance-leak" method

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[1]Specific activity 55.2 mc/mM, Amersham/Searle, Des Plaines, Illinois.

[2]J. T. Baker Chemical Co., Phillipsburg, New Jersey.

[3]Kindly furnished by Dr. E. W. Bird, Iowa State University.

[4]Atlas Chemical Industries, Inc., Wilmington, Delaware.

with a vibrating reed electrometer[1] and recorded as millivolts per hour on an automatic recorder.[2] The equipment was standardized by using 2% CO_2 in air which contained 0.7 μc ^{14}C/liter, so that the area under the curve could be used as a direct measure of the radioactivity expired. The curve was traced onto paper[3] of uniform weight, and the weight of the inscribed area was used to determine the area under the curve. These values were converted to μc expired per unit of time.

NADP-linked enzyme systems

Preparation of homogenate A 1 gm sample of tissue was homogenized in 3 ml cold 0.25 M sucrose for 1 minute. An additional 5 ml of sucrose solution was added and the mixture was homogenized for 2 minutes. The volume was made to 10 ml. The homogenate was then centrifuged at 26,500 x g at 4°C for 30 minutes. The clear supernatant fraction was removed by pipette in such a way that contamination with the sediment of the overlying lipid layer was avoided. The homogenates for HMPD and IDH activity determinations were stored at -20°C until assayed. The MDH activity was assayed on the day of autopsy.

Measurement of enzyme activity The change in optical density at 340 mu produced by the reduction of NADP over a 5

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[1]Model 31, Applied Physics Corporation, Monrovia, Calif.

[2]Brown Electronik Recorder, Minneapolis-Honeywell Regulator Co., Philadelphia, Pennsylvania.

[3]Albanene, Iowa State University Bookstore.

minute interval at 25°C was used as an index of activity for each of the enzymes. Concentration of the enzyme was such that the change in optical density was linear over this period of time. Solutions containing appropriate cofactors and buffer were placed in the cuvette and the reactions were initiated by the addition of the appropriate substrate, G-6-P, L-malate or dl-isocitrate. It was found in preliminary investigation that some homogenates and cofactors reacted to produce a change in optical density without addition of the substrate so samples were routinely incubated for 5 minutes before addition of the substrate. Blank determinations without added NADP were made for each sample by the same procedure. In addition blanks, omitting the substrate but with NADP present, were run periodically to check for interference. The system of Glock and McLean (1953) as modified by Thorp (1966) was used to measure the combined activity of the hexose monophosphate shunt dehydrogenases. The method of Ochoa (1955a) was used for determining IDH activity. Fitch and Chaikoff's (1961) modification of Ochoa's (1955b) method for determining MDH activity was used. Glucose-6-phosphate, L-malate, and dl-isocitric acid were Sigma grade.[1] The NADP was NRC grade.[2] The components of the systems used for measuring the activity of the enzymes are shown in Table 7.

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[1]Sigma Chemical Company, St. Louis, Missouri.

[2]General Biochemicals, Chagrin Falls, Ohio.

Table 7. Assay systems of NADP-linked enzymes

	HMP	IDH	MDH
		μmoles	
NADP	.25	.135	.135
$MgCl_2$	5.00	-	-
$MnCl_2$	-	1.800	3.000
Glycyl-glycine[a]	125.00	75.000	75.000
G-6-P	.50	-	-
dl-isocitrate	-	.600	-
L-malate	-	-	5
		ml	
Homogenate	.1-1.0	.05-1.0	.1-1.0
Total volume	2.5	3.0	3.0

[a]HMPD-pH 7.6, IDH and MDH - pH 7.4.

Acetate-1-^{14}C incorporation into fatty acids

Preparation of homogenates The homogenates were prepared in the same manner as were homogenates for measuring the NADP-linked enzymes with one exception. The homogenation solution for FAS consisted of a potassium phosphate-bicarbonate buffer, pH 8.00 (K_2HPO_4-.085 M, KH_2PO_4-.009 M, $KHCO_3$-.07 M). Homogenates were stored under nitrogen at -20°C for 3 months before being assayed.

Incubation system A modification of the system of Harlan and Wakil (1963) was used. The components of the incuba-

tion mixture are listed in Table 8.

Table 8. Components of system for acetate-1-^{14}C incorporation into fatty acids

	μmoles
Sodium acetate (4 μc/μmole)[a]	.50
NADPH, NRC[b]	1.00
NADH, NRC[b]	1.00
ATP, NRC[b]	4.00
Coenzyme A (approx. 85%)[b]	.08
Glutathione reduced form[c]	10.00
DL-isocitrate[c]	20.00
$MnCl_2$	2.00
Potassium phosphate buffer (pH 6.5)	150.00
Sodium bicarbonate	16.00
	mg
Bovine serum albumin[b]	3
Cytosol protein[d]	1-2
Total volume, 1 ml	

[a]Amersham/Searle, Des Plaines, Illinois.

[b]General Biochemicals, Chagrin Falls, Ohio.

[c]Sigma Chemical Company, St. Louis, Missouri.

[d]26,500xg supernatant protein here and throughout thesis.

In preliminary investigations to determine the metal requirements it was found that manganese was a better cofactor than magnesium (Table 9).

Table 9. Effect of metals on acetate-1-^{14}C incorporation into fatty acids

Metal		^{14}C Incorporation		
$MgCl_2$	$MnCl_2$	Experiment I	Experiment II	Experiment III
μmoles			cpm	
2	-	10		
-	0.8	26		
2	0.8	16		
-	0.6		48	
-	1.0		90	
0.4	0.6		70	
-	1.0			239
-	1.5			354
-	2.0			364
-	2.5			362

In Experiment I, 0.8 μmoles $MnCl_2$ permitted 2-1/2 times as much incorporation as 2 μmoles $MgCl_2$. This increase in activity was only 1-1/2 times if 2 μmoles $MgCl_2$ were added in addition to the $MnCl_2$. To determine if this apparent inhibition by $MgCl_2$ was due to concentration or an effect of the metal itself, Experiment II was run. When $MnCl_2$ was increased from 0.6 μmole to 1 μmole, incorporation of ^{14}C into fatty acids was increased 60%, however, when 0.4 μmole $MgCl_2$ was added the activity was decreased 20%. Therefore $MnCl_2$ seemed to be a better cofactor than $MgCl_2$. To establish the optimum level of manganese, Experiment III was run. Here the optimum activity was obtained with 2 μmoles of $MnCl_2$. A dependence on protein concentration was also seen in preliminary work. When protein

concentrations were low it was impossible to obtain a linear curve with increasing levels of homogenate. The specific activity (cpm/mg cytosol protein) increased with increasing levels of protein. Adding 3 mg of bovine serum albumin to the incubation system stabilized the system. Figure 1 shows a linear response over the range of concentration of cytosol protein that was used in the assays.

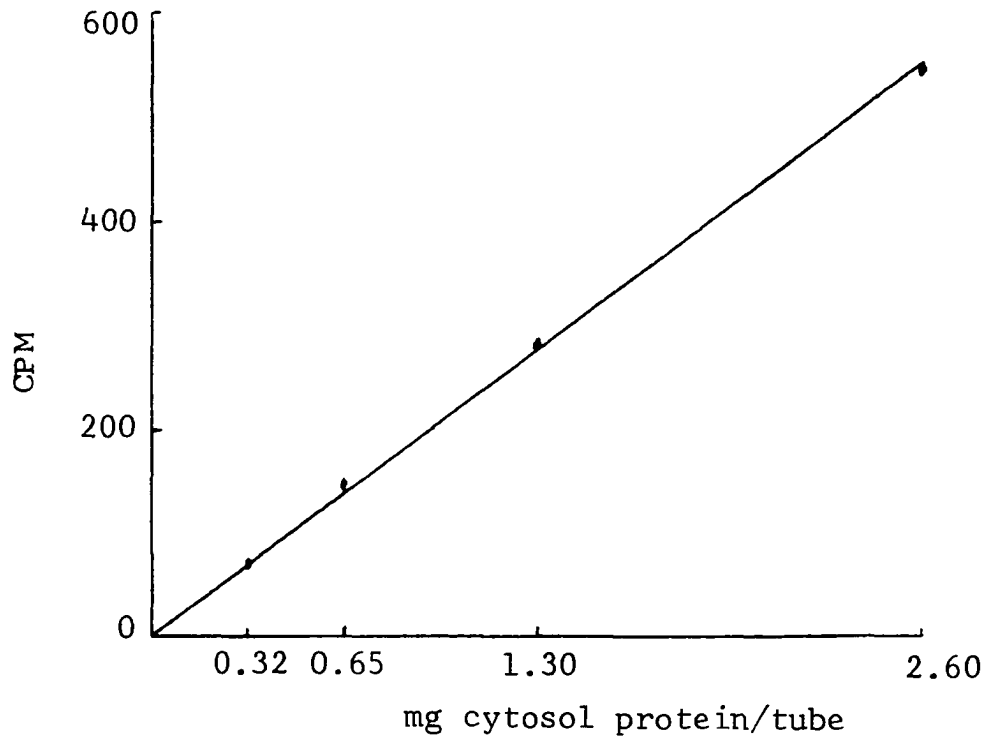

Figure 1. Acetate-1-^{14}C incorporation into fatty acids with 3 mg BSA added per tube

The effect of varying other components was of less importance. Addition of isocitrate to the system gave an 8% increase in activity with 20 μmoles while further addition did not increase the activity. When bicarbonate was added in concentrations

greater than 40 μmoles it became inhibitory.

Incubation The reaction was started by adding the homogenate and was carried out in capped pyrex tubes. These tubes were incubated in a constant temperature water bath at 37°C for 1 hour. The reaction was linear over a period of at least 1-1/2 hours. The reaction was stopped by adding 1 ml of alcoholic potassium hydroxide (KOH, 10 g; 70 ml CH_3OH/ 100 ml). To saponify the triglycerides, tubes were heated in a water bath for 30 minutes at 80°C.

Extraction and counting The samples were acidified with 0.3 ml of 12 N sulfuric acid. Two ml of pentane were added to the tubes and they were shaken vigorously for 10 seconds, and then centrifuged at 5,000 x g for 5 minutes. The pentane layer was removed and this extraction was repeated 4 times. The extracts were combined, washed with 10 ml of deionized water, then made to volume of 10 ml. An aliquot was plated and counted as previously described.

Protein determinations

Supernatant protein was determined by the biuret method (Gronall et al., 1949) using a Technicon Autoanalyzer.[1]

[1] N14B, Technicon Corp., Chauncey, New York.

Statistical Methods

The standard errors of the mean and Duncan's Test on differences between means were determined according to methods described by Steel and Torrie (1960). Analysis of variance and regressions were determined by methods described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The present study was conducted in two experiments, which were identical in design and dietary variable. They differed only in treatment before sacrifice and in parameters measured. In Experiment 1, animals were fasted 12±1 hours of the last day of the refeeding trial, after that they received 1 gram of their respective diets. One hour later they were injected intraperitoneally with 10 μc of palmitate-1-^{14}C and placed in a metabolism chamber for four hours. The animals were then anesthetized by intraperitoneal injection of sodium pentobarbital and livers and epididymal tissues removed for analyses of lipid and fatty acid content as well as incorporation of palmitate-1-^{14}C into the lipid fractions. Animals in Experiment 2 were fasted 12±1 hours prior to sacrifice by stunning and partial decapitation. Activities of the combined hexose monophosphate shunt dehydrogenases, NADP-malate dehydrogenase, and NADP-isocitrate dehydrogenase were measured in the soluble supernatants from liver and epididymal tissue. In addition acetate-1-^{14}C incorporation into fatty acids by the soluble supernatant fraction of the liver was determined.

Body weights

In period 1, length of depletion period, rate of weight loss and feed consumption in both experiments were similar to results obtained in previous studies in this laboratory (Stadler, 1969; Kopec, 1969). The first 100 g of body weight

was lost at the rate of 2.8 g per day. Restriction of food intake accelerated the rate of loss of the second 100 g to 5 g per day. Similar responses to depletion in the present study and previous studies permit comparisons between the various studies.

Confirming earlier findings in this laboratory (Stadler, 1969; Kopec, 1969) inclusion of fat into low protein diets increased the rate of gain (Tables 10 and 11). Inclusion of fat into the diet also improved ($P < 0.01$) the rate of gain when adequate protein was in the diet, but had no effect when protein was excessive. Some significant differences were shown with increasing levels of dietary protein, but no definite pattern emerged.

In Experiment 1, caloric intake was increased when fat was included in low and adequate protein diets. In Experiment 2, this influence was also seen when protein was excessive. In contrast, dietary protein decreased ($P < 0.01$) the caloric intake. The low level of protein did not permit optimal conversion of food energy to body weight gain, groups III and VI. Increasing dietary protein from low to adequate levels markedly improved ($P < 0.01$) the food efficiency. However, the food efficiency was not consistently affected by a further increase in protein above adequate levels.

Table 10. Body weights and energy intake during realimentation-experiment 1

Group[1]		Initial weight g	Final weight g	g gain/day	Energy intake kcal/day	Feed efficiency g gain/100 kcal
III	OF P	293±1[2]	353±3[a,3]	6.3±.2[a]	68.4±2.3[a]	9.2±.2[a]
IV	OF3P	294±1	365±2[b]	7.4±.2[b]	61.9±1.3[b]	12.0±.1[b]
V	OF9P	295±1	364±1[b]	7.3±.4[b]	55.6±1.8[c]	13.0±.3[c]
VI	2OF P	293±1	375±3[c]	8.6±.2[c]	83.6±2.6[d]	10.3±.2[d]
VII	2OF3P	294±1	378±5[c]	8.9±.5[c]	69.2±2.0[a]	12.7±.5[c,b]
VIII	2OF9P	295±1	367±2[b]	7.6±.2[b]	56.6±1.7[c]	13.3±.4[c]
			Results of A. O. V. (P<)			
P		NS	.05	.05	.01	.01
F		NS	.01	.01	.01	.01
P X F		NS	.01	.01	NS	NS

[1]10 animals per group.

[2]Mean±standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

Table 11. Body weights and energy intake during realimentation-experiment 2

Group[1]		Initial weight g	Final weight g	g gain/day	Energy intake kcal/day	Feed efficiency g gain/100 kcal
III	OF P	295±1[2]	361±3[a,3]	7.0±.3[a]	72.1±2.2[b]	9.6±.3[a]
IV	OF3P	294±1	370±2[b,c]	8.0±.2[b]	67.0±1.3[c]	11.9±.3[b]
V	OF9P	293±1	365±3[b,c]	7.6±.3[b,c]	48.3±1.1[e]	12.8±.3[b]
VI	20F P	294±2	372±2[a]	8.2±.3[b]	82.2±2.2[a]	9.9±.2[a]
VII	20F3P	294±1	380±3[a,b]	9.1±.3[a]	74.8±1.4[b]	12.0±.3[b]
VIII	20F9P	292±2	363±3[c]	7.4±.3[b,c]	58.9±1.7[d]	12.5±.3[b]
			Results of A. O. V. (P<)			
P		NS	.01	.01	.01	.01
F		NS	.05	.01	.01	NS
P X F		NS	.05	NS	.05	NS

[1]15 animals per group.

[2]Mean±standard error.

[3]Means with the same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

Respiratory ^{14}C

Animals were injected with 10 μc palmitate-1-^{14}C and respiratory ^{14}C was monitored for the following four hours. The four hours were divided into 5 time periods for interpretation of results: 0-0.5 hrs, 0.5-1.0 hrs, 1.0-2.0 hrs, 2.0-3.0 hrs, and 3.0-4.0 hrs (Table 12). Analysis of variance of the refeeding groups, including time as a variable, indicate a significant effect of time ($P<.01$). In all groups oxidation rates increased for approximately one hour then decreased throughout the remainder of the four hour period. The control stock group oxidized more ($P<.01$) palmitate-1-^{14}C at each time period than the depleted or refed animals.

Of the two dietary variables, only fat exerted a significant effect on the total amount of palmitate-1-^{14}C oxidized during the 4 hour period. Inclusion of fat in the diet increased ($P<.05$) the total respiratory ^{14}C when the diet contained excessive protein. Statistical analyses of each time period showed that this difference due to diet occurred in the second and third hours. In the second hour animals receiving diet 20F9P had significantly higher ($P<.05$) rates of oxidation than all other groups subjected to refeeding. In the third hour group 20F9P had higher ($P<.05$) rates of oxidation than group 0F9P. After adjusting for differences in body weight, the higher oxidation rate with diet 20F9P was still apparent. Why inclusion of fat into the high protein diet would cause this higher oxidation rate and not affect the oxidation rate

Table 12. Respiratory $^{14}CO_2$ in mμc

4 hours after injection of 10 μc palmitate-1-^{14}C

Group[1]		Hours after injection 0.0.5	0.5-1	1-2	2-3
I	CS	517±38[a,2,3]	928±42[a]	1299±65[a]	554±26[a]
II	CR	244±16[b]	576±56[b]	677±50[c]	266±14[c]
III	OF P	317±24[b]	585±35[b]	629±57[c]	272±25[c]
IV	OF3P	436±58[b]	657±51[b]	708±57[c]	316±22[b,c]
V	OF9P	351±22[b]	551±21[b]	625±36[c]	300±20[c]
VI	2OF P	497±64[b]	704±49[b]	696±40[c]	286±15[c]
VII	2OF3P	410±44[b]	660±65[b]	699±51[c]	312±17[b,c]
VIII	2OF9P	373±34[b]	659±54[b]	894±54[b]	364±45[b]

[1]10 animals per group.

[2]Mean±standard error.

[3]Means with the same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

[4]P=1.075 g protein/100 kcal.

3-4	Cumulative mμc	Cumulative mμc/100 g BW
308±15[a]	3607±150[a]	738±27[a,4]
180±16[b]	1941±125[c]	666±40[a,b,c]
181±13[b]	1984±132[c]	576±36[c]
197±11[b]	2314±162[b,c]	653±44[a,b,c]
191±1[b]	2017± 56[c]	572±16[c]
164±8[b]	2348±129[b,c]	636±32[a,b,c]
187±11[b]	2268±161[b,c]	610±42[b,c]
194±12[b]	2483±101[b]	695±31[a,b]

with P and 3P is not immediately obvious. Differences in body lipid pools and amount of palmitate in these lipids would seem to be the most likely cause. However, as will be discussed later, Groups V and VIII do not seem to differ in these respects to any greater extent than do Groups IV and VII, which do not differ in oxidation rates. Perhaps this high oxidation rate of Group VIII is related to the fact that this diet contains very little carbohydrate, only 12% of the calories. With glucose availability at a minimum, a shift toward fatty acids as the energy source may have occurred.

Liver

Tissue weights

Hepatic weights are presented in Table 13. In experiment 1, average hepatic weight for control stock animals was 13.1 g. During depletion this value was reduced to 6.7 g. After refeeding, the weight was 67 to 77% that of stock controls. Liver weight increased ($P<.01$) when fat was included in the diet and protein was low or adequate. Increasing protein from adequate to excessive amounts in low fat diets also caused an increase in liver weight (groups IV and V). In experiment 2, responses to dietary treatments differed from those in the first experiment. Inclusion of fat in the diet only increased ($P<.05$) the weight of the liver when protein was adequate, whereas in experiment 1 this effect was also exerted when protein was low. In both experiments, increasing dietary protein

Table 13. Weights of hepatic tissue

Group[1]		Experiment 1 g	Experiment 2 g
I	CS	13.1±.3[a,2,3]	12.1±.6[a]
II	CR	6.7±.2[b]	6.1±.2[b]
III	OF P	8.8±.2[c]	9.6±.2[c]
IV	OF3P	8.9±.2[c]	8.9±.2[d]
V	OF9P	9.8±.2[d]	9.7±.2[c]
VI	2OF P	9.9±.4[d]	9.9±.3[c]
VII	2OF3P	9.6±.2[d]	9.6±.2[c]
VIII	2OF9P	10.0±.2[d]	9.3±.2[c,d]
		Results of A. O. V. (P<)	
P		.05	NS
F		.01	NS
P X F		NS	NS

[1]Experiment 1, 10 animals per group. Experiment 2, Group I-4 animals, Group II-8 animals, Groups III-VIII-15 animals.

[2]Mean±standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

from adequate to excessive amounts caused an increase ($P < .05$) in liver weight when low fat diets were used.

Hepatic lipids

Livers from animals in the control stock group contained 590 mg of total lipid or 4.6% (Table 14). After dietary restriction the lipid content was reduced to 216 mg or 3.2%. Thus the extent of hepatic lipid loss on dietary restriction exceeded that of the combined non-lipid components of the tissue. After refeeding all treatments produced an increase in total hepatic lipid. A mild fatty infiltration noted when protein was low disappeared when protein was raised to adequate or excessive levels. Increased triglyceride formation, decreased triglyceride removal by lipoproteins, decreased fatty acid oxidation, decreased turnover of phospholipid and loss of mitochondrial integrity have been suggested as possibly causes of fatty livers (Glenn et al., 1963). Lee (1967) working in this laboratory with chronically malnourished rats found an increase in fatty acid oxidase activity when protein was raised from low to adequate and excessive amounts. Therefore, a decrease in fatty acid oxidase activity could have been a contributing factor in the accumulation of hepatic lipids with suboptimal protein nutriture (Groups III and VI).

The concentration of nonphospholipid did not significantly change during dietary restriction (Table 14). During refeeding the elevation in total hepatic lipids observed in animals fed

Table 14. Hepatic total lipid and nonphospholipid

Group		Total lipid mg	Total lipid % of liver	Nonphospholipid mg	Nonphospholipid % of liver	Nonphospholipid % of lipid
I	CS	590±32[a,1,2]	4.6±.3[a]	219±30[a]	1.7±.2[a]	36±4[a]
II	CR	216±10[b]	3.2±.1[b]	81± 8[b]	1.2±.1[a]	37±3[a]
III	OF P	543±40[a]	6.2±.5[c]	335±40[c]	3.8±.5[b]	60±3[b]
IV	OF3P	346±10[c]	3.9±.4[a,b]	142±25[a,b]	1.6±.3[a]	40±4[a]
V	OF9P	343±15[c]	3.5±.1[a,b]	108±11[b]	1.1±.1[a]	31±2[a]
VI	20F P	652±72[a]	6.4±.6[c]	440±18[d]	4.3±.5[b]	66±3[b]
VII	20F3P	425±20[c]	4.4±.2[a]	199±15[a]	2.1±.2[a]	47±3[c]
VIII	20F9P	377±24[c]	3.8±.2[a,b]	129±15[a,b]	1.3±.1[a]	34±2[a]
		Results of A. O. V. (P<)				
P		.01	.01	.01	.01	.01
F		.01	NS	.01	NS	.05
P X F		NS	NS	NS	NS	NS

[1]Mean ± standard error.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

low protein diets was reflected in this fraction. In contrast, the concentration of phospholipids decreased during dietary restriction, but was not affected by either fat or protein in the refeeding diets (Table 15).

Table 15. Hepatic phospholipid

Group		mg	% of liver	% of lipid
I	CS	376± 7[a,1,2]	2.9±.2[a]	64±3[a]
II	CR	137± 7[b]	2.0±.1[b]	64±3[a]
III	OF P	191± 5[c]	2.2±.1[b]	37±3[b]
IV	OF3P	195±14[c]	2.2±.2[b]	58±3[a,c]
V	OF9P	238±11[c,d]	2.4±.1[b,d]	70±2[a]
VI	2OF P	230±18[c,d]	2.3±.1[b,d]	37±3[b]
VII	2OF3P	231±16[c,d]	2.4±.1[b,d]	54±2[c]
VIII	2OF9P	259±13[d]	2.6±.1[d,a]	70±3[a]
		Results of A. O. V. (P<)		
P		NS	NS	.01
F		.01	.01	NS
P X F		NS	NS	NS

[1]Mean ± standard error.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

Fatty acid composition of hepatic nonphospholipid

During dietary restriction the relative concentrations of palmitic (C16), palmitoleic (C16:1), and stearic acids (C18) increased (Table 16). An increase was also observed in a fatty acid with chain length longer than 20 carbons. This fatty acid has not been identified and will be referred to as C > 20:4 throughout this report (Table 17). The percentages of linoleic (C18:2) and linolenic (C18:3) acids decreased during the depletion period.

Palmitic, palmitoleic, and oleic acids were greatly elevated over control values when diet OFP was fed. The combined value for these fatty acids was 85% as compared to 43% for group CS (Table 17). Concurrently linoleic and arachidonic acids decreased. These alterations in the fatty acid pattern suggest heightened lipogenesis. When protein was increased to adequate or excessive levels, the percentages of C16, C16:1, C18:1 were decreased ($P < .01$), but these fatty acids were still elevated over control values. The combined percentages of C16, C16:1, C18:1 was 62% for group IV and 58% for group V as compared to 43% for group I. In addition to a reduction in fatty acids indicative of de novo synthesis, increasing protein from low to adequate or excessive amounts caused changes in the distribution among the polyunsaturated fatty acids. Dietary protein may have stimulated the desaturation and elongation enzymes, since arachidonate increased from 2 to 9% and C > 20:4, which was virtually absent when the dietary protein

Table 16. Fatty acid composition of hepatic nonphospholipids

Group	Fatty acids as percent of hepatic nonphospholipids C14	C16	C16:1	C18
I	0.8± 0[a,1,2]	21.5±0.1[a]	1.8±.1[a]	9.4±.2[ab]
II	1.5±.1[ab]	24.8±0.1[ab]	2.8±.1[a]	12.4±.4[a]
III	1.6± 0[ab]	34.1±0.6[c]	8.7±.1[b]	5.1±.1[c]
IV	1.8± 0[ab]	27.3±1.5[b]	6.5±.1[c]	8.5±.4[b]
V	2.3±.1[b]	27.2±0.2[b]	5.7±.1[c]	11.4±.2[ab]
VI	1.0± 0[a]	25.8±0.1[ab]	2.3± 0[a]	4.8±.1[c]
VII	0.7± 0[a]	22.3±0.2[a]	1.6± 0[a]	8.0±.7[bc]
VIII	1.3±.2[ab]	22.0±0.7[a]	1.5±.2[a]	12.5±.4[a]
	Results of A. O. V. (P<)			
I vs. II	NS	.05	.05	.01
P	NS	.01	.01	.01
F	.01	.01	.01	NS
P X F	NS	NS	.01	NS

[1]Mean ± standard error, standard errors reported as 0 are less than 0.05.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

Table 17. Fatty acid composition of hepatic nonphospholipids

	Fatty acids as percent of hepatic nonphospholipids				
Group	C18:1	C18:2	C20:4	C>20:4	C16 + C16:1 +C18:1
I	19.7±.2^{a}	29.6±0.4^{de}	13.6±0.4^{ab}	0.6±0.1^{c}	43
II	18.0±.9^{a}	20.3±1.0^{c}	14.0±0.7^{ab}	5.3±0.6^{a}	46
III	42.4±.5^{b}	6.0±0.2^{a}	2.1±0.1^{d}	trace	85
IV	30.7±.9^{c}	11.9±0.4^{b}	8.6±1.5^{bc}	4.4±1.1^{ab}	62
V	25.8±.5^{c}	13.1±0.3^{b}	9.7±0.5^{bc}	4.3±0.8^{ab}	58
VI	25.6±.2^{c}	35.9±0.4^{f}	trace	trace	54
VII	18.7±.8^{a}	34.6±1.7^{ef}	11.9±1.7^{ab}	1.5±0.3^{bc}	42
VIII	18.5±.7^{a}	27.5±3.0^{d}	16.1±2.0^{a}	0.3±0.1^{c}	42
	Results of A. O. V. (P<)				
I vs. II	NS	.01	NS	.01	
P	.01	NS	.01	.01	
F	.01	.01	.01	.01	
P X F	.01	.01	NS	.05	

was low, comprised 4.4% of the fatty acids.

With diet 20FP only oleic acid was elevated over control values. This relative rise in oleic acid is probably not an indication of increased lipogenesis since palmitic and palmitoleic acids were not elevated. Oleic acid is the end product of palmitic acid desaturation and elongation. An increase in this acid could be expected if transport of lipid out of the liver were inhibited. By the same reasoning the fact that with adequate or excessive protein levels the elevation in oleic acid was obliterated could have been the result of a lipotropic action of protein on liver lipids. Here, as when the low fat diets were fed, a redistribution of the polyunsaturated fatty acids was seen when protein was increased from low to adequate or excessive amounts. Both arachidonate and C >20:4 were virtually absent when protein was low. Arachidonate now contributed 12 to 16% of the fatty acids.

Fatty acid composition of hepatic phospholipids

The phospholipids of the liver are structural lipids, thus the fatty acid composition of these lipids was not greatly influenced by dietary restriction (Table 18). Here as in the nonphospholipid fraction, palmitic, palmitoleic, and oleic acids were elevated ($P < .01$) over control values when low fat diets were used combined values being 25 and 40%, respectively. Only small changes were noted due to increasing levels of dietary protein. With diet 20FP palmitic acid was elevated

Table 18. Fatty acid composition of hepatic phospholipids

Group	Fatty acids as percent of hepatic phospholipids C16	C16:1	C18	C18:1
I	15.9±.1[a,1,2]	0.2± 0[a]	26.6±.2[d]	8.9± 0[c]
II	20.2±.1[c]	0.6± 0[b]	23.1±.1[ab]	9.3± 0[c]
III	23.9±.2[e]	3.6± 0[d]	22.6±.1[a]	13.0±.1[e]
IV	22.1±.1[d]	3.3± 0[cd]	24.1±.3[bc]	12.4± 0[d]
V	21.7±.1[d]	3.3± 0[c]	24.3±.2[bc]	13.4±.1[e]
VI	20.3±.1[c]	0.3± 0[a]	25.1±.1[c]	6.8±.1[b]
VII	17.7±.1[b]	0.2± 0[a]	27.6±.4[d]	6.0± 0[a]
VIII	15.3±.1[a]	trace	27.9±.4[d]	7.2± 0[b]
	Results of A. O. V. (P<)			
I vs. II	.01	NS	.01	NS
P	.01	NS	.01	.01
F	.01	.01	.01	.01
P X F	.01	NS	NS	NS

[1]Mean ± standard error, standard errors reported as 0 are less than 0.05.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

C18:2	C20:4	C>20:4
13.1±.1[a]	29.8±.2[a]	5.3± 0[a]
16.0±.1[b]	19.1±.1[b]	11.6±.1[d]
8.6±.1[c]	16.0±.2[c]	12.2±.1[d]
10.2±.1[d]	16.1±.1[c]	11.7±.1[d]
10.7±.1[e]	16.0±.1[c]	10.6±.2[c]
15.5±.1[b]	23.4±.1[d]	8.6±.1[b]
15.5±.1[b]	25.2±.2[e]	7.8±.1[b]
17.6±.1[f]	26.5±.1[f]	5.2±.1[a]
.01	.01	.01
.01	.01	.01
.01	.01	.01
NS	.01	.05

over that of group CS, 20.3 vs. 15.9%. However, the percentage of oleic acid was less ($P < .01$) than control values, so the combined percentage of C16, C16:1, and C18:1 was 27% as compared to 25% in group CS. Increasing the level of dietary protein to adequate or excessive levels decreased the percentage of palmitic acid, so that combined values of C16, C16:1, and C18:1 were 24% for group VII and 22% for group VIII.

Previous work in this laboratory was confirmed in that the fatty acids of the phospholipids indicative of de novo synthesis were elevated when OFP diet was fed and that the fatty acid pattern with diet 20FP differed little from the pattern exhibited in control animals (Stadler, 1969; Kopec, 1969). These patterns characteristic of the low protein diets showed few changes with increasing levels of protein used in the present experiment.

Palmitate-1-^{14}C incorporation into hepatic lipids

Both groups of control animals, CS and CR, incorporated approximately 5% of the injected dose into hepatic lipids (Table 19). Amount of lipid, amount of palmitate, metabolic size of the animal, rate of oxidation of fatty acids are factors which might have influenced the assimilation of palmitate-1-^{14}C into tissue lipids. The rate of oxidation or metabolic size of the animal did not seem to influence the assimilation of palmitate-1-^{14}C into hepatic lipids. Control stock animals had a higher rate of oxidation based on respiratory $^{14}CO_2$ than

Table 19. Palmitate-1-^{14}C incorporation into hepatic lipids

Group	Total lipid mμc/tissue	Total lipid mμc/g lipid	Nonphospholipids mμc/tissue	Nonphospholipids mμc/g lipid	Phospholipids mμc/tissue	Phospholipids mμc/g lipid
I	522±40[a,1,2]	892± 66[d]	199±26[b]	987±111[a]	301±22[abc]	807± 53[a]
II	523±56[a]	2532±388[a]	169±32[b]	2159±384[b]	367±63[a]	2948±780[b]
III	509±59[a]	976±113[bcd]	280±56[a]	894±150[a]	228±18[d]	1197±312[cd]
IV	418±30[b]	1238± 24[b]	143±20[b]	1101±132[a]	250±19[cd]	1296± 63[cd]
V	402±27[b]	1180± 82[bcd]	119±13[b]	1112± 58[a]	331±42[ab]	1404±184[d]
VI	359±12[b]	621± 48[e]	170±19[b]	406± 29[c]	223±12[d]	993± 43[a]
VII	392±30[b]	940± 79[cd]	151±20[b]	762± 83[a]	224±18[d]	1001± 84[ac]
VIII	445±34[b]	1220±140[bc]	118±17[b]	920± 81[a]	288±22[bcd]	1109± 56[acd]
			Results of A. O. V. (P<)			
P	NS	.01	.01	.01	.01	NS
F	NS	.05	NS	.01	NS	.01
P X F	.05	NS	.05	NS	NS	NS

[1]Mean ± standard error.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

control restricted animals, but total radioactivity incorporated into hepatic lipids was identical in the two groups. However, when incorporation of the tracer was expressed per g lipid, a marked difference was seen between groups CS and CR. The specific activity of hepatic lipids of group CS was approximately one-third that of group CR. This result would be expected on the basis of large differences in hepatic lipid between stock and restricted controls, causing considerable dilution of the tracer in control stock animals. Incorporation of ^{14}C from palmitate into total hepatic lipid with diet OFP was higher ($P < .05$) than that found with other refeeding diets. This may be related to the greater amount of palmitate in hepatic lipid of group OFP. All other refeeding groups had less incorporation into total lipid. Specific activity values reflect the decrease in total lipid with increasing levels of protein.

The amount of palmitate-1-^{14}C incorporated into the nonphospholipids ranged from 1 to 3%. With diet OFP 2.8% of the injected dose was incorporated into the nonphospholipids, while values for controls and other refed groups were lower ($P < .05$). As indicated earlier this is probably due to a larger amount of palmitate which accompanied the mild fatty infiltration of livers of these animals.

All animals except group III incorporated more palmitate-1-^{14}C into the phospholipid than the nonphospholipid fraction. The incorporation of ^{14}C into the phospholipid fraction seems

to follow the same pattern as the accumulation of phospholipids, (Table 19) that is a tendency to increase with increasing levels of dietary protein.

Hepatic NADP-linked enzymes

NADPH is one of the major cofactors required for the de novo synthesis of fatty acids and also for the elongation and desaturation of long chain fatty acids. The activities of two hepatic enzyme systems producing NADPH were correlated to lipogenesis in several studies. The activities of these enzymes, hexose monophosphate shunt dehydrogenases and malic enzyme, have increased in fasted, refed rats which were shown to be in a state of heightened lipogenesis. In studies in this laboratory (Thorp, 1966 and Lee, 1967) with chronically malnourished rats, the activity of HMPD showed the "overshoot" typically observed when fasted rats are refed with high carbohydrate diets. Also the depression of the HMPD activity with inclusion of dietary fat, characteristic of the response of fasted refed rats was noted in these studies. Furthermore after a 3 day refeeding period, depression of hepatic HMPD activity occurred when excessive protein was substituted for carbohydrate in the refeeding diet. One of the objectives of the present experiment was to see if these changes in HMPD activity were sustained for a longer period of time and if other hepatic NADPH producing enzymes and lipogenesis reflected the same responses to refeeding. Hepatic hexose monophosphate

shunt activity, NADP-malate dehydrogenase and isocitrate dehydrogenase activities were measured. Enzyme activity has been expressed per gram of tissue and per 100 mg supernatant protein. In the subsequent discussion changes in activity are based on 100 mg supernatant protein unless otherwise specified.

Hexose monophosphate shunt dehydrogenases As stated previously, workers in this laboratory (Thorp, 1966; Lee, 1967) found HMPD activity elevated over control values when chronically malnourished rats were refed low fat diets. Although we did not measure HMPD activity of the control animals, it is assumed that this is the case in the present experiment, since responses of animals to depletion were quite similar to the previous experiments (Thorp, 1966; Lee, 1967).

Higher levels of protein in the low fat diet promoted higher activity (Table 20). McDonald and Johnson (1965) also reported a relationship of dietary protein to the level of shunt activity. They suggested that the inducer of the refeeding diet was carbohydrate but that an adequate level of dietary protein was necessary to replenish the amino acid pool to a level which would allow this rate of synthesis. This explanation would seem to fit the data of the present experiment. Animals had been severely depleted of total nitrogen reserves; upon refeeding protein synthesis for tissue regeneration might have first priority for available amino acids. This could limit the amino acids available for synthesis of high amounts of HMPD, if these enzymes are not needed. This explanation is

Table 20. Hepatic hexose monophosphate shunt dehydrogenases

Group[1]		HMPD activity ΔOD/min/g tissue	ΔOD/min/100 mg protein
III	OF P	8.28±0.74[b,2,3]	5.64±0.56[a]
IV	OF3P	10.91±1.23[a,b]	7.07±0.71[b]
V	OF9P	11.34±1.67[a]	8.10±1.16[c]
VI	2OF P	3.48±0.76[c]	2.26±0.46[d]
VII	2OF3P	3.73±0.56[c]	2.41±0.38[d]
VIII	2OF9P	2.19±0.31[c]	1.34±0.18[e]
		Results of A. O. V. (P<)	
P		NS	NS
F		.01	.01
P X F		NS	.05

[1]Ten animals per group.

[2]Mean ± standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

less adequate for the data obtained in an earlier study from this laboratory. After 3 days of refeeding Lee (1967) obtained the highest degree of overshoot in HMPD activity with the intermediate protein level. Values were approximately 3, 9, and 6 fold over control values with low, adequate, and excessive protein, respectively. In other words she saw a depression with excessive protein, while there is no indication of a similar response after the 10 day refeeding period in the present study. Why the HMPD activity with diet OF9P would be

greater relative to HMPD activity with diet OF3P after 10 days than 3 days of refeeding is unknown. Perhaps the induction of HMPD lagged in the animals fed excessive protein. It would be of considerable interest to study the time course of events related to this activity. It would also be of interest to know if and when the HMPD activity returns to values found in stock controls.

As has been shown by many investigators, HMPD activity was depressed with fat containing diets. Furthermore, activity decreased ($P<.05$) when protein was raised from adequate to excessive levels, groups VII and VIII. This finding is similar to that of earlier studies with a 3 day refeeding period (Lee, 1967), but differs from the enzymatic response to low fat diets in the present study. The ratio of calories from dietary carbohydrate to calories from carbohydrate plus fat has been implicated as an important factor in determining activity of some of the enzymes related to lipogenesis (Baldwin, 1966). This principal could apply to the present experiment. The carbohydrate to carbohydrate plus fat ratios were similar (0.59 and 0.54) with diets containing low and adequate protein. However, when protein contributed 51% of the calories, the ratio was much lower, 0.24, and the activity of HMPD was also lower, than with the other protein containing diets.

NADP-malate dehydrogenase With low fat diets NADP-malate dehydrogenase was elevated (Table 21). The activity of MDH was approximately 7 times the control value when dietary protein was low, group III. The elevation over control values was only 4 and 2 fold when protein was adequate or excessive, respectively, and they were not statistically different.

With the addition of dietary fat, values of MDH were lower but the depression of MDH activity with protein was still apparent when lactalbumin was increased from low to adequate amounts, groups VI and VII. The values for MDH activity were not higher than the control value with the 20F3P and 20F9P diets.

The apparent suppression of the induction of NADP-malate dehydrogenase by increasing dietary protein may not be an effect of dietary protein per se, but a consequence of reducing dietary carbohydrate, since protein was added to the diet at the expense of carbohydrate. A logarithmic relationship existed between MDH activity and the % of calories contributed by carbohydrate (Figure 2). This correlation is quite high, $r=0.97$.

NADP-isocitrate dehydrogenase Hepatic IDH did not show as great a response to refeeding as the previous NADP-linked enzyme system (Table 22). Values for IDH activity for low fat groups were 150-190% of the control values. With adequate dietary protein the elevation over the control value

Table 21. Hepatic NADP-malate dehydrogenase

Group[1]		MDH actitity ΔOD/Min/g tissue	ΔOD/min/100 mg protein
I	CS	1.06±0.21[d,2,3]	0.74±.17[d]
III	OF P	7.93±1.09[a]	5.30±.74[a]
IV	OF3P	4.58±0.72[b]	3.07±.54[b]
V	OF9P	2.80±0.34[c]	2.03±.27[c]
VI	2OF P	3.15±0.39[c]	2.09±.26[b,c]
VII	2OF3P	1.39±0.23[d]	0.92±.16[d]
VIII	2OF9P	0.73±0.15[d]	0.44±.08[d]
		Results of A. O. V. (P<)	
P		.01	.01
F		.01	.01
P X F		NS	.01

[1]Four animals in Group I, 10 animals in Groups III-VIII.

[2]Mean ± standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

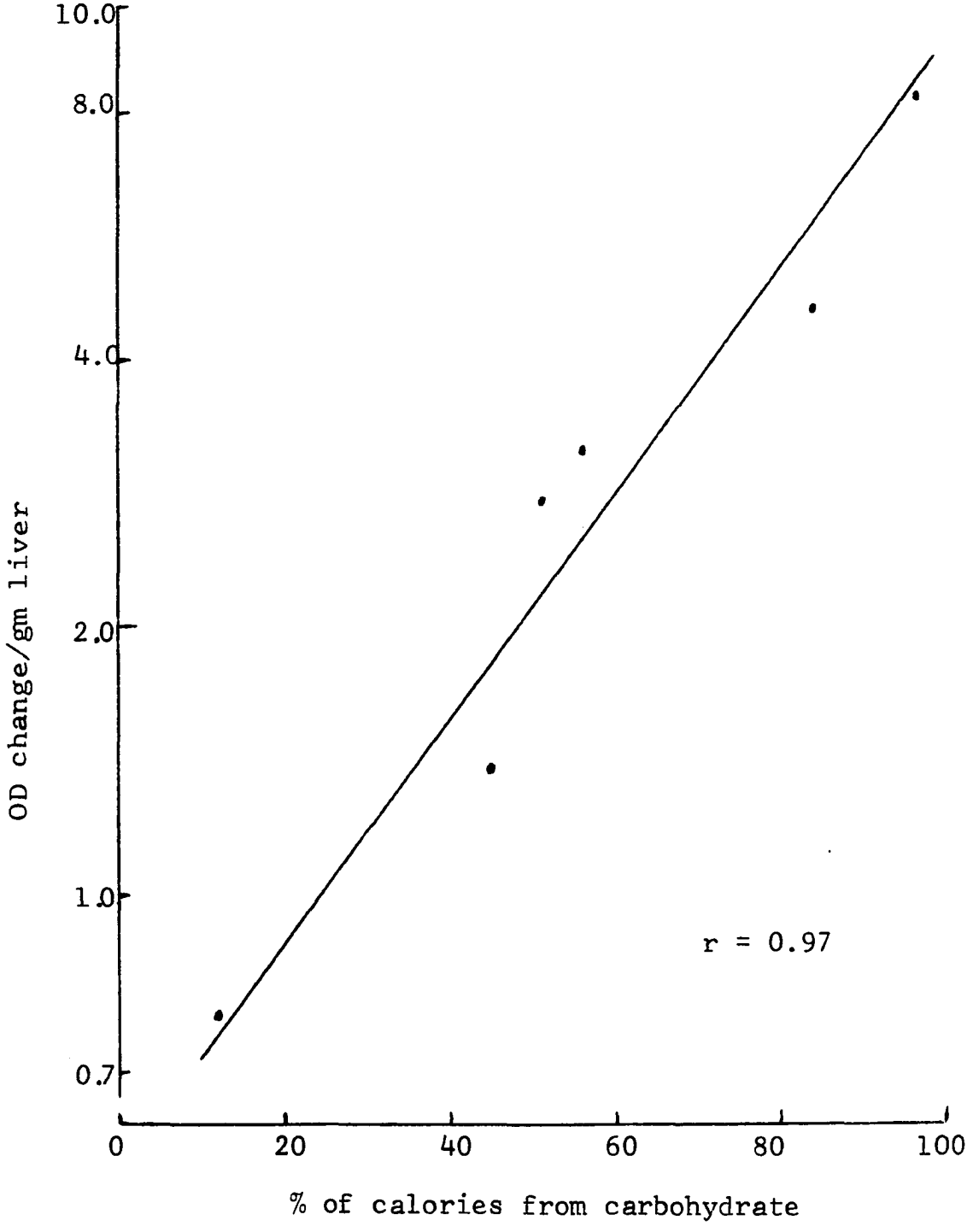

Figure 2. Correlation of dietary calories from carbohydrate and hepatic MDH activity

Table 22. Hepatic NADP-isocitrate dehydrogenase

Group[1]		IDH activity ΔOD/min/g tissue	ΔOD/min/100 mg protein
I	CS	14.9±2.2[c,2,3]	9.7±0.9[b]
III	OF P	22.4±1.4[b]	14.6±0.9[a,b]
IV	OF3P	30.4±1.8[a]	19.2±1.7[a]
V	OF9P	20.9±3.8[b,c]	14.9±2.6[a,b]
VI	20F P	22.9±3.0[b]	14.6±2.0[a,b]
VII	20F3P	24.5±1.7[a,b]	15.7±1.4[a]
VIII	20F9P	22.5±3.6[b]	14.2±2.3[a,b]
		Results of A. O. V. (P<)	
P		NS	NS
F		NS	NS
P X F		NS	NS

[1]Four animals in Group I, 6 animals in Groups III-VIII.

[2]Mean ± standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

was significant (P<.05).

In contrast to the other two NADP-linked enzyme systems, IDH activity was not affected by the inclusion of fat in the diet. Here as in groups III to V, IDH activity of animals fed adequate protein was significantly elevated over control values.

Acetate-1-^{14}C incorporation into fatty acids

Acetate-1-^{14}C incorporation into fatty acids was measured in vitro as an indicator of fatty acid synthesis (FAS). A decrease in FAS during the restriction period was expected as this had been described for fasted refed, young rats (Table 23). However, FAS was unchanged after the period of severe protein-calorie restriction. Differences in the method of depletion, acute vs. chronic starvation, may account for the fact that FAS was not decreased during depletion. Another difference between the present study and those with fasted refed rats is the age of the animals. Mature rats were used in the present study whereas younger animals have been used in the studies with fasted refed rats. Gellhorn et al. (1962) reported a decline in FAS of adipose tissue during aging. Perhaps FAS decreases in hepatic tissue also with age, so that dietary restriction produces no further decline.

The incorporation of acetate-1-^{14}C was elevated ($P<.05$) approximately 600% over control values with diet OFP. In this respect the chronically malnourished rats used in this experiment responded to refeeding similarly to fasted refed rats. Tepperman et al. (1968) have reported "hyperlipogenesis" with a zero protein refeeding diet, so it appears that the enzymes involved in lipogenesis are not dependent on a continuing exogenous supply of amino acids.

A depression of acetate-1-^{14}C incorporation was found when protein was raised from low to adequate amounts in fat

Table 23. Acetate-1-^{14}C incorporation into fatty acids

Group[1]		Acetate-1-^{14}C incorporation into fatty acids cpm/g tissue x 10^5	cpm/mg protein x 10^3
I	CS	5.03±2.57[b,c,2,3]	3.27±1.69[b,c]
II	CR	4.03±1.80[c]	2.78±1.16[b,c]
III	OF P	23.78±7.06[a]	19.56±3.99[a]
IV	OF3P	14.02±4.89[a,b]	10.31±4.41[b]
V	OF9P	15.65±4.63[a,b]	11.15±2.85[b]
VI	2OF P	8.74±3.83[b,c]	6.42±2.88[b,c]
VII	2OF3P	3.43±0.86[c]	2.56±1.66[b,c]
VIII	2OF9P	2.14±0.56[c]	1.46±0.29[c]
		Results of A. O. V. (P<)	
P		NS	.05
F		.01	.01
P X F		NS	NS

[1]Four animals in Group I, 8 animals in Groups II-VIII.

[2]Mean ± standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

free diets. Reduced rate of lipogenesis by dietary protein has been reported by Kimura (1969). This decrease in lipogenesis was linked to the decrease in carbohydrate, based on the fact that in subsequent studies protein stimulated lipogenesis less than carbohydrate. If this would apply in the

present experiment, depression in FAS would be expected when protein was further increased to excessive amounts in low fat diets. However, this was not the case. The depression of FAS when protein was increased from low to adequate amounts seems to be due to the increase in protein per se and not to a decrease in carbohydrate. The age and the nutritional status of the animals in the present experiment differ greatly from those used in other studies reported in the literature. Perhaps the nutritional status of these animals is the fact that causes a different pattern of response in FAS due to dietary protein. As mentioned earlier these animals had been depleted of their protein stores. With refeeding, they need to synthesize protein for tissue regeneration. With the low protein diet the amount of protein synthesis may be limited by the supply of amino acids. If this were the case, then less energy would be needed for protein synthesis and therefore would be stored as fat. With more amino acids made available with the adequate or excessive protein diets, protein synthesis could proceed at a higher rate and less energy would be converted to fat.

Relative to comparable groups on low fat diets, acetate-1-^{14}C incorporation was reduced with fat at all levels of protein intake. These values did not differ statistically from those of controls. Increasing dietary protein from low to adequate amounts decreased the acetate-1-^{14}C incorporation from 6.42 to 2.56. However, this decrease was statistically

insignificant due to the large variability among animals. Here again, it would be of interest to know the time course of the enzymes adaptation to the refeeding diet. This trend toward a difference in FAS between groups fed low and adequate protein may have been more exaggerated at another time period.

The mean acetate-1-^{14}C incorporation per g tissue was significantly correlated with mean HMPD and MDH activities. The correlation was higher between FAS and MDH activities ($r=.93$) than FAS and HMPD activities ($r=.75$). The combined percentages of C16, C16:1, and C18:1 in the NPL fraction of hepatic lipids was also significantly correlated ($P<.01$) to the mean acetate-1-^{14}C incorporation, $r=.90$.

Adipose Tissue

Tissue weights

Tissue weights for experiments 1 and 2 are given in Table 24. The epidiymal fat pad weighed 6.9 g at the beginning of the depletion period (group I). After period 1, when body weight had been reduced by 40% of the initial weight, the epididymal fat pads weighed 1.6 g, a 77% reduction from the initial weight. After refeeding, the epididymal fat pad was restored to 50 to 60% of its original weight. Analysis of variance showed no significant differences between refeeding groups. In experiment 2, weights of the epididymal fat pads from the control stock and control restricted animals were quite similar to those of the same group in experiment 1. In

experiment 2, high fat diets produced significantly heavier fat pads than did low fat diets when protein was low or adequate. Since food efficiency was identical on comparable protein levels (Table 11), increase in weight of the fat pads may be related to the higher caloric intake of animals receiving fat in the diet. A similar trend was noted in experiment 1, but differences could not be verified statistically.

Epididymal lipid

Adipose tissue serves as the major site of energy storage in the animal body. A portion of the energy presented to the adipose tissue for storage is in the form of glucose or amino acids or their metabolic products. Adipose tissue by the process of de novo synthesis of fatty acids converts these compounds to a high caloric density energy source. The adipose tissue also processes triglycerides for storage. These triglycerides may be from chylomicrons from ingested fats or lipoproteins from the liver. Therefore the amount and composition of the lipid in the adipose tissue is a reflection of all these processes.

Table 24. Weights of epididymal fat pads

Group[1]		Experiment 1 g	Experiment 2 g
I	CS	6.9±.5[a,2,3]	6.1±.7[a]
II	CR	1.6±.3[b]	1.8±.4[b]
III	OF P	3.6±.4[c]	2.7±.2[c]
IV	OF3P	3.7±.2[c]	2.6±.1[c]
V	OF9P	3.7±.4[c]	2.5±.1[c]
VI	20F P	4.3±.2[c]	3.5±.3[d]
VII	20F3P	3.9±.3[c]	3.6±.3[d]
VIII	20F9P	3.5±.2[c]	3.0±.2[c]
		Results of A. O. V. (P<)	
P		NS	NS
F		NS	.01
P X F		NS	NS

[1]Experiment 1, 10 animals per group. Experiment 2, Group I-4 animals, Group II-8 animals, Groups III-VIII-15 animals.

[2]Mean ± standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

The epididymal fat pads from the control stock animals contained 68% lipid (Table 25). This value was reduced to 35% by the end of the depletion period. After 10 days of refeeding, approximately 50 to 60% of the initial lipid was recovered. Increasing dietary protein from low to adequate and excessive levels did not significantly affect the weight or the concentration of lipid of the adipose tissue. However, the dietary protein level affected the response of epididymal lipid concentration to dietary fat. As noted by other workers in this laboratory (Stadler, 1969; Kopec, 1969), when dietary protein was low the inclusion of fat in the diet significantly increased ($P<.05$) the concentration of epididymal lipid from 56 to 63%. This effect of dietary fat was not apparent when protein was adequate or excessive. Why the difference in lipid concentrations between groups III and VI is significant is unclear since larger differences (20% compared to 6%) in tissue weight and lipid weight, upon which these values were based, were not statistically significant. Experimental variations in opposite directions in tissue weights or lipid weights or both could make this statistical observation possible.

Fatty acid composition of epididymal lipid

The means for fatty acid concentration of epididymal lipid are presented in Table 26. During the period of dietary restriction prior to refeeding, changes in the relative percentage of fatty acids were small. Some of these changes were

Table 25. Epididymal lipid

Group		Epididymal lipid (%)	(g)
I	CS	68±2[a,1,2]	4.7±.3[a]
II	CR	35±6[b]	0.4±.3[b]
III	OF P	56±2[c]	2.1±.3[c]
IV	OF3P	60±2[c,d]	2.2±.2[c]
V	OF9P	60±2[c,d]	2.3±.2[c]
VI	20F P	63±1[a,d]	2.7±.2[c]
VII	20F3P	61±2[c,d]	2.4±.2[c]
VIII	20F9P	61±1[c,d]	2.2±.2[c]

[1]Mean ± standard error.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

Table 26. Fatty acid composition of epididymal lipid

Group	Fatty acids as percent of epididymal lipid C14	C16	C16:1	C18
I	0.8± 0[a,1,2]	17.5±.1[a]	3.3± 0[ab]	3.2± 0[cd]
II	1.0± 0[b]	14.7±.1[b]	2.6± 0[c]	4.9±.1[a]
III	2.2± 0[d]	25.4±.5[c]	11.6±.4[d]	3.0± 0[de]
IV	2.3± 0[d]	22.9±.4[d]	10.0±.4[e]	3.2± 0[cd]
V	2.7± 0[e]	23.6±.4[d]	8.7±.3[f]	3.6± 0[b]
VI	1.0± 0[b]	16.0±.1[e]	3.6± 0[b]	2.5± 0[f]
VII	1.1± 0[b]	15.8± 0[e]	3.1± 0[b]	2.8± 0[ef]
VIII	1.4± 0[c]	15.8± 0[e]	2.4± 0[c]	3.5± 0[bc]
	Results of A. O. V. (P<)			
I vs. II	NS	.01	NS	.01
P	.01	NS	.01	.01
F	.01	.01	.01	.01
P X F	NS	NS	NS	NS

[1]Mean ± standard error, standard errors reported as 0 were less than 0.05.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

C18:1	C18:2	C18:3	C16+C16:1 +C18:1
30.0±.1[a]	40.6±.2[a]	4.6± 0[a]	50.8
34.0±.1[b]	39.7±.3[a]	2.8± 0[b]	51.3
37.4±.3[c]	18.6± 0[b]	1.9± 0[ce]	74.4
37.9±.4[c]	21.5±.4[b]	2.0± 0[c]	70.8
37.1±.2[c]	22.0± 0[b]	2.3± 0[d]	69.4
31.3±.1[d]	44.0±.1[c]	1.5± 0[f]	50.9
31.2±.1[d]	44.5±.1[c]	1.6± 0[ef]	50.1
31.7±.1[d]	43.4±.2[c]	1.8± 0[ce]	49.9
.01	NS	.01	
NS	NS	.01	
.01	.01	.01	
NS	NS	NS	

statistically significant ($P<.01$), but probably not physiologically important. The percentages of palmitate (C16) and linolenate (C18:3) were decreased and the percentages of stearate (C18) and oleate (C18:1) increased.

Confirming previously presented data, refeeding diet OFP resulted in large increases ($P<.01$) in palmitic acid and its desaturation and elongation products, palmitoleic and oleic acid. The combined value for C16, C16:1 and C18:1 was 74% for this group compared to 51% for group I. The percentage of linoleic acid was decreased ($P<.01$) as would be expected, since it cannot be synthesized by animal tissue. These changes in fatty acid composition are characteristic of hyperlipogenesis. The fatty acid pattern was similar when protein was fed at adequate or excessive levels, however the elevation in palmitic and palmitoleic acids was less pronounced.

When fat was included in the diet, only small changes from control values were noted in concentrations of C16, C16:1, and C18:1. Changes in individual acids were not in the same direction, so that the combined percentages of palmitic, palmitoleic, and oleic acids differed less than 1% from the control values. Therefore from the percentages of fatty acids it would seem that there is no response in fatty acids indicative of de novo synthesis as a result of increasing dietary protein. However, dilution by dietary fat could obscure changes in absolute amounts of newly synthesized fatty acids. That this may be the case is evident when the weight of the fatty acids

is estimated (Table 27). The combined weight of C16, C16:1, and C18:1, is 1.24 g with diet 20FP while the corresponding value is only 1.08 g with the adequate protein diet.

A small but consistent increase in stearic acid was found with each increase in protein level in both 0F and 20F diets. This change in stearic acid was significant ($P<.01$) between adequate and excessive protein levels and could possibly be indicative of a stimulation of de novo synthesis of fatty acids by the microsomal system or of a shift in the elongation and desaturation pathway of palmitic acid to oleic acid. However, since this change in stearic acid concentration is small it may be merely a reflection of changes in other fatty acids. That the latter is probably the case can be seen from the approximate weight of this fatty acid (Table 27). Values were .06, .06, and .07 g for groups III, IV, and V, respectively. The weight of oleic acid did not always increase with increased protein, groups VII and VIII.

In general increasing the level of protein in the refeeding diet had little effect on the fatty acids that denote changes in de novo synthesis or in the incorporation of fatty acids from dietary fat.

Palmitate-1-^{14}C incorporation into epididymal lipid

Data of palmitate-1-^{14}C incorporation into epididymal lipid are presented in Table 28. Approximately 2 to 5% of the injected dose of palmitate-1-^{14}C was incorporated into the

Table 27. Estimated weight of fatty acids in epididymal lipids[1]

Group		Total	Estimated grams of fatty acids C16	C16:1	C18	C18:1	C18:2	C16+C16:1+ C18:1
I	CS	4.24	.74	.14	.14	1.27	1.72	2.15
II	CR	0.36	.05	.01	.02	0.12	0.14	0.18
III	OF P	1.90	.48	.22	.06	0.71	0.35	1.41
IV	OF3P	1.99	.46	.20	.06	0.71	0.43	1.37
V	OF9P	2.08	.49	.18	.07	0.77	0.46	1.44
VI	20F P	2.44	.39	.09	.06	0.76	1.07	1.24
VII	20F3P	2.17	.34	.07	.08	0.67	0.97	1.08
VIII	20F9P	1.99	.31	.05	.07	0.63	0.86	0.99

[1]Calculated by the method of Lyman et al. (1964), mean weight percent of fatty acid x 0.95 x 0.95 x mean total lipid.

Table 28. Palmitate-1-^{14}C incorporation into epididymal lipids

Group		mμc/tissue	mμc/g lipid
I	CS	201± 31[c,1,2]	54±17[a]
II	CR	394± 55[b]	1095±258[b]
III	OF P	510±103[a]	246±26[c]
IV	OF3P	503± 55[a]	232±31[c]
V	OF9P	515± 56[a]	224±54[c]
VI	20F P	527± 59[a]	193±22[c]
VII	20F3P	343± 55[b]	154±28[c]
VIII	20F9P	362± 36[b]	173±19[c]
	Results of A. O. V. (P<)		
P		.01	NS
F		.01	NS
P X F		.01	NS

[1]Mean ± standard error.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

epididymal lipid. Here in contrast to hepatic lipids, the incorporation of palmitate-1-^{14}C seems to be influenced by the rate of oxidation. Animals from the control stock group incorporated less (P<.01) label into epididymal lipid than did animals from the depleted or refed groups. Both dietary protein and fat exerted a significant influence (P<.01) on the assimilation of radioactive palmitate into epididymal lipid and this influence was dependent on the other dietary con-

stituent as indicated by a significant protein-fat interaction. Animals fed diets containing adequate or excessive protein, VII and VIII, had less incorporation of ^{14}C into epididymal lipid than animals from other refed groups. This is probably due to a relatively low level of palmitate. When these data were expressed as mμc/g epididymal lipid, animals from the control groups reflected the differences in lipid pools. Control stock animals, having larger lipid pools, had very low specific activity, while control restricted animals had less lipid causing a larger specific activity.

Epididymal NADP-linked enzymes

Hexose monophosphate shunt dehydrogenases With low fat refeeding regimes no differences were observed due to dietary protein (Table 29). With the inclusion of fat in the diet the activity was not affected when dietary protein was low, groups III and VI. The failure of dietary fat to suppress the induction of HMPD was unexpected. However, in the studies reporting suppression of epididymal HMPD activity by dietary fat, the level of dietary protein had been adequate (Leveille, 1967b, 1967c; Konishi, 1966; Fabry et al., 1970). The suppression of HMPD activity when protein was raised from low to adequate amounts in 20F diets is harder to explain. Since dietary fat did not suppress the activity of the shunt enzymes when substituted for carbohydrate in the low protein diets, explanations such as slowing of metabolites over meta-

Table 29. Epididymal hexose monophosphate shunt dehydrogenases

Group		HMPD activity ΔOD/min/g tissue	HMPD activity ΔOD/min/100 mg protein
III	OF P	4.31±2.9[a,b,1,2]	14.3±3.1[a,b,c]
IV	OF3P	5.80±1.0[a]	22.7±4.2[a]
V	OF9P	4.21±0.6[a,b]	20.3±3.8[a,b]
VI	20F P	5.37±2.2[a]	25.1±9.7[a]
VII	20F3P	1.46±0.2[b,c]	6.9±1.3[b,c]
VIII	20F9P	0.86±0.1[c]	3.8±0.4[c]
		Results of A. O. V. (P<)	
P		NS	NS
F		.01	NS
P X F		.05	.01

[1]Mean ± standard error.

[2]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

bolic pathways or changes in carbohydrate content would not seem to be the complete answer. One suggestion might be that there is a specific level of dietary carbohydrate necessary for optimum stimulation of this enzyme, and that it is this level of carbohydrate which is governing the induction of this enzyme rather than changes in other dietary constituents.

The HMPD activity and lipogenesis, as reflected by total lipid and fatty acid composition, seem to be well correlated in animals fed low fat diets. However, this is not the case

with fat containing diets. The decrease in HMPD activity seen when protein was increased from low to adequate amounts is not accompanied by a significant decrease in total lipid or the fatty acids that denote de novo synthesis.

NADP-malate dehydrogenase The elevation in MDH in refed groups over controls, 10 to 100 fold, are larger than values found in the literature, 8 to 10 fold increases over control values (Leveille, 1967b; Young et al., 1964) (Table 30). However, animals in those experiments were either not fasted or fasted for 2 to 4 days before feeding high carbohydrate diets. Therefore, the larger changes from control values seen here may be a consequence of the chronic malnutrition which preceded refeeding. The report of Young et al. (1964) that a relationship exists between the duration of fasting and the degree and duration of induction of malic enzyme upon refeeding seems to substantiate this idea.

With low fat diets the level of protein did not significantly affect the activity of epididymal NADP-MDH. Though, here as was the case with HMPD, values tended to be higher when protein was fed at an adequate level. Suppression of MDH activity by dietary fat was evident, with animals fed adequate or excessive levels of protein with fat having lower ($P<.05$) values than those receiving the low fat diets. NADP-malate dehydrogenase has been reported to be closely related to the carbohydrate of the diet and we found this to be true with the hepatic enzyme. However, the epididymal enzyme does

Table 30. Epididymal NADP-malate dehydrogenase

Group[1]		MDH activity ΔOD/min/g tissue	ΔOD/min/100 mg protein
I	CS	0.04±0.01[a,2,3]	0.3±0.1[a]
III	OF P	8.41±1.32[b]	28.8±6.1[b,c]
IV	OF3P	11.59±2.24[b]	45.8±9.8[b]
V	OF9P	8.04±1.5[b]	39.2±2.0[b]
VI	20F P	3.01±0.89[c]	13.0±1.1[c,d]
VII	20F3P	1.59±0.27[c]	6.9±1.1[d]
VIII	20F9P	0.90±0.13[c]	3.9±0.5[d]
		Results of A. O. V. (P<)	
P		NS	NS
F		.01	.01
P X F		NS	NS

[1]Four animals in Group I, 10 animals in Groups III-VIII.

[2]Mean ± standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

not seem to be directly related to changes in the carbohydrate content of the diet.

NADP-isocitrate dehydrogenase The activity of IDH was low in adipose tissue compared to liver (Table 31). Some elevation of the activity of this enzyme was produced when low fat diets with low and adequate protein were refed. With excessive protein in the low fat diets this elevation was not statistically different from the control values. The elevation

Table 31. Epididymal NADP-isocitrate dehydrogenase

Group[1]		IDH activity ΔOD/min/g tissue	IDH activity ΔOD/min/100 mg protein
I	CS	0.05±.004[c,2,3]	0.41±.11[c]
III	OF P	0.52±.08[a,b]	1.71±.22[a,b]
IV	OF3P	0.68±.10[a]	2.54±.38[a]
V	OF9P	0.51±.04[a,b]	1.34±.41[b,c]
VI	20F P	0.31±.16[b]	0.98±.03[b,c]
VII	20F3P	0.24±.07[b]	1.06±.28[b,c]
VIII	20F9P	0.23±.08[b]	1.06±.40[b,c]
		Results of A. O. V. (P<)	
P		NS	NS
F		.01	.01
P X F		NS	NS

[1]Four animals in Group I, 6 animals in Groups III-VIII.

[2]Mean ± standard error.

[3]Means with same superscripts are not different at the 5% level by Duncan's Multiple Range Test.

of values for low fat groups over control values may indicate that this enzyme furnished some of the NADPH for adaptive hyperlipogenesis.

Mean MDH activity expressed per g tissue was better correlated with that of IDH, $r=0.99$, than with that of HMPD, $r=0.75$. The correlation of the activity of these enzyme systems to lipogenesis is unclear. MDH, IDH and probably HMPD activities were elevated when low fat diets were refed. Furthermore changes in the fatty acid composition had indicated a high level of lipogenesis. The enzyme systems did not respond in the same manner to high fat diets as did the fatty acid pattern. HMPD was just as high when dietary protein was low as with the low fat diets. Furthermore, the activity of MDH was not significantly depressed when fat was added to the low protein diets. But considerable decreases in percentages of C16, C16:1, and C18:1 were seen when fat was added to the diet. Dilution of fatty acids by exogenous fat could obliterate changes in percentages of the fatty acids of de novo synthesis. The fatty acid composition is a reflection of changes over a ten day period of time, whereas the enzymes were measured only on day 10. To elucidate the significance of the elevation of these enzymes and their relation to lipogenesis in the epididymal fat pad of chronically malnourished rat, studies are needed which measure changes in the enzymes throughout the refeeding period. A more direct means of measuring lipogenesis such as acetate-1-^{14}C incorporation into fatty acids would

also be helpful. A double isotope technique labeling both the C and H of glucose, malate, or isocitrate would also be useful in supplying information on how important these large increases in NADPH producing enzymes are to adaptive hyperlipogenesis.

General Discussion

In previous experiments in this laboratory inclusion of fat into low protein diets had been found to accelerate the animals' rate of gain and increase their caloric intake. An increase in the relative amount of redeposition of lipid in epididymal tissue with fat in the diet was a consequence of these overall changes (Stadler, 1969; Kopec, 1969). These effects of fat were confirmed in the present experiment. However, when dietary protein was increased to adequate or excessive levels, inclusion of fat into the refeeding diet did not affect rate of gain, caloric intake, or the percent of epididymal lipid. These findings indicate that the low level of protein used in these diets, though adequate for maintenance of nitrogen balance of adult healthy rats may be insufficient for optimum recovery under the particular conditions of our experimental design. The increase in food efficiency noted when protein was increased from low to adequate levels lends support to this idea.

Confirming previous work in this laboratory, refeeding diets containing the low level of protein produced mild fatty

infiltration of hepatic tissue. This accumulation of lipid was mainly reflected in the nonphospholipid fraction of hepatic lipids. Nonphospholipids of animals fed diet OFP contained high concentrations of palmitic, palmitoleic, and oleic acids. Increases in these fatty acids have been correlated with heightened lipogenesis in fasted refed animals (Allmann et al., 1965a). Such would seem to be the case in the present experiment with chronically malnourished rats since acetate-1-^{14}C incorporation into fatty acids was elevated some 6 times over control stock values.

The fatty infiltration which accompanied low protein diets disappeared when protein was increased to adequate levels. In animals fed low fat diets decreases in palmitic acids as well as its elongation and desaturation products were also noted with the increase in dietary protein. In accordance with the theory that decreases in concentrations of these fatty acids indicate decreased lipogenesis, acetate-1-^{14}C incorporation into fatty acids with diet OF3P was depressed. It would be tempting to infer from these data that increased lipogenesis is the cause of fatty livers that result from low protein diets. However, the fatty acid pattern of the NPL fraction seen with diet 20FP does not indicate that hepatic lipogenesis had been elevated to any great extent. An increase in oleic acid was noted but no significant increases were seen in palmitic or palmitoleic acids. In looking at these hepatic fatty acid patterns it is important to take into consideration

the dilution of newly synthesized fatty acids by those entering the body pool from the diet. Therefore the amounts of palmitic, palmitoleic and oleic acids could be present in much larger quantities even though the percentages are not elevated. Acetate-1-^{14}C incorporation into hepatic fatty acids of group 20FP was twice that of the stock control, but was statistically not different. When dietary protein was increased to 3P in the fat containing diets neither the percentage of oleic acid nor acetate-1-^{14}C incorporation were elevated over CS values. Although an increase in lipogenesis may be a contributing factor in production of fatty livers, it would by no means seem to be the prime factor. Decrease in fatty acid oxidase and triglyceride removal by lipoproteins have been suggested as other possible causes of fatty livers (Glenn *et al.*, 1963).

As mentioned earlier, Lee (1967) had noted an increase in hepatic fatty acid oxidase with increasing levels of protein after 3 days of refeeding. This worker used octanoate as the substrate in her *in vitro* studies. In the present experiment where fatty acid oxidation was measured *in vivo* utilizing palmitic acid as a substrate, protein had no effect. If this discrepancy is not due to the difference in length of refeeding period, it may indicate that the liver is more sensitive to changes in dietary protein than are other tissues. Another explanation might be that palmitic acid is not oxidized at the same rate as other fatty acids. The level of protein did influence the effect exerted by dietary fat. Dietary fat

increased ($P<.05$) the rate of oxidation of palmitate-1-^{14}C when included in the high protein diets. This increase in palmitate oxidation is possibly a result of the low amount of carbohydrate in the diet, with a shift toward fatty acids as the energy source.

We have not studied changes in lipoprotein with increasing levels of dietary protein, and therefore have no reason to believe that a decrease in removal of triglycerides by lipoproteins is not a contributing factor to fatty infiltration of the liver observed on our low protein diets.

Another interesting finding in the present experiment was the large increases in the concentrations of arachidonic acid and the fatty acid C>20:4 with the increase in protein from low to adequate levels. This increase in relative concentrations of the more polyunsaturated fatty acids may be due to stimulation of the elongation and desaturation systems by dietary protein. A recent report by De Gomez Dumm et al. (1970) supports this suggestion. These workers contribute an increase in linoleic desaturation in fasted refed rats to dietary protein. Whether a breakdown in the desaturation and elongation system of fatty acid synthesis is in some way involved in the production of fatty livers is unknown. Studies of the microsomal system responsible for desaturation and elongation of linoleic acid as affected by low and adequate protein would be most useful.

The metabolic origin of the lipid in fatty livers has not been settled. Two of the possibilities that have been considered are that lipid is formed by the liver or that the lipid is formed by adipose tissue and that free fatty acids from this site are converted into glycerides in the liver which are then unable to escape at an adequate rate (Macdonald, 1966). If the source of this lipid were adipose tissue, one might expect that either adipose tissue would contain less lipid or show an increased rate of lipogenesis in presence of fatty livers. From our data there is no indication that this is the case. Increasing dietary protein had no appreciable effects on either the amount or the fatty acid composition of epididymal lipid.

Further increase of dietary protein to excessive levels did not seem to influence hepatic lipid accumulation, fatty acid patterns of hepatic lipids or acetate-1-^{14}C incorporation into fatty acids to any great extent.

The production of NADPH was at one time considered to be one of the prime regulators of fatty acid biosynthesis. Positive correlations between lipogenic capacity and HMPD and malic enzyme activities were found (Tepperman and Tepperman, 1958a; 1964). That the elevation in lipogenesis precedes the induction of HMPD and malic enzyme activities showed that increased production of NADPH was possibly a result rather than a cause of heightened lipogenesis (Tepperman and Tepperman, 1961; Leveille, 1970).

The elevation in acetate-1-^{14}C incorporation with diet OFP was accompanied by heightened activities of MDH and IDH. Though we did not assay HMPD activity in control animals, data from previous experiments would indicate that HMPD activity was also elevated. The rise in IDH activity was slight in comparison to MDH activity but it was approaching significance at the $P<.05$ level. The contribution of reducing equivalents from IDH could be significant, since activity was already high in group CS. However, caution must be used in making quantitative comparisons between enzymes since the assay conditions may not be equally favorable to all the enzymes.

A change in dietary protein from low to adequate amounts in absence of fat did not produce coordinated changes in the hepatic enzymes. Of the three enzymes studied only MDH activity showed a depression with the increase in dietary protein as had acetate-1-^{14}C incorporation. In contrast, HMPD activity increased whereas lipogenesis based on acetate-1-^{14}C decreased. With the increase in protein to excessive levels in fat free diets, a further increase in HMPD activity was noted. Likewise, MDH activity continued to decrease with the increase in protein. In this instance neither enzyme activity seems to be well correlated with lipogenesis, since lipogenesis as measured by acetate-1-^{14}C incorporation was not affected by increasing dietary protein from adequate to excessive amounts in low fat diets. From these data it seems possible that protein was the inducer of hepatic HMPD activity upon refeeding.

However we cannot generalize from these data since this relationship does not hold when fat was included in the diet. Here where HMPD activity had been depressed by inclusion of fat in the diet, no change was noted when protein was raised from low to adequate levels, and a depression occurred when protein was further increased to excessive levels. Therefore it would appear that the induction of hepatic HMPD in chronically malnourished rats is dependent on both protein and carbohydrate. This speculation is supported by the work of Potter and Ono (1961) with fasted refed animals.

Furthermore a time factor may also play a part, Lee (1967) found a depression in HMPD activity with excessive protein levels after a 3 day refeeding period. This emphasizes the complexity of interpreting results obtained after one fixed period of refeeding. Perhaps changing dietary constituents also alters the time course of induction of this enzyme.

Our data suggests that hepatic malic enzyme activity in chronically malnourished rats is induced by the carbohydrate in the diet, while a dependency of MDH on adequate dietary protein was not demonstrated. The highest activity of MDH was observed with diet OFP even though this level of protein seems inadequate for recovery of normal lipid metabolism of the liver. Of the three NADP-linked enzymes studied malic enzyme demonstrates the best correlation with lipogenesis as measured by acetate-1-^{14}C incorporation. Whether this correlation is meaningful is unknown. MDH activity appeared to reflect

changes in the carbohydrate content of the diet. From our data the decreases observed in lipogenesis cannot be attributed to decreases in carbohydrate content alone, as there is no depression of acetate-1-^{14}C incorporation into fatty acids when protein is increased from adequate to excessive levels in the low fat diets.

The epididymal NADP-linked enzymes were strikingly different from the hepatic enzymes in some respects. Malic enzyme activity was higher in epididymal tissue than in hepatic tissue. In addition the epididymal MDH was elevated over control values to a much greater extent than the hepatic enzyme. Epididymal HMPD and MDH activities did not respond to changes in the protein content of low fat diets. Perhaps the liver modulates the flow of substrates so that the supply of glucose and amino acids to the adipose tissue is not significantly influenced by dietary changes. Another possibility is that the refeeding period was not long enough for a dietary influence on adipose tissue. However, the latter explanation does not seem adequate since dietary fat depressed the activity of the enzymes in some cases. HMPD did not respond to dietary fat when the level of protein in the refeeding diet was low, as did the hepatic enzyme. However, HMPD activity was depressed when protein was increased to adequate levels in fat containing diets. Epididymal HMPD activity then would seem to be dependent on a specific level of carbohydrate in the diet. No differences in HMPD activity are observed due to changes in

the diet as long as the carbohydrate content is high, that is roughly 50% or more of the diet.

Isocitrate dehydrogenase activity was low in this tissue in comparison to hepatic tissue. In contrast to the hepatic enzyme, the epididymal enzyme responded to changes in dietary constituents, being depressed when fat was included in the adequate protein diet.

Although we did not measure lipogenesis in the adipose tissue by acetate-1-^{14}C incorporation, comparisons of NADP-linked enzymes to lipogenesis can be made to a limited extent, utilizing the accumulation and composition of the epididymal lipid. With the exception of HMPD and MDH activity with the 20FP diet, the epididymal NADP-linked enzymes seem to be well correlated with lipogenesis as indicated by the fatty acid pattern. The fatty acids associated with lipogenesis were decreased when fat was included in the diet and changed to no great extent with changes in dietary protein.

From our data it appears that the hepatic NADP-linked enzymes are more sensitive to changes in the protein content of the refeeding diet than are the epididymal NADP-linked enzymes. Changes in activity of HMPD are not always correlated to changes in other parameters measuring lipogenesis. Malic enzyme activity is better correlated with other measures of lipogenesis in both hepatic and adipose tissue than are HMPD and IDH activity.

SUMMARY

The purpose of this study was to determine the effects of dietary protein on lipid metabolism of adult rats. The experimental model used was refeeding adult rats that had been subjected to chronic depletion of lipid and protein reserves. The objectives of the study were to determine the effect of variations in dietary fat and protein on indices of lipid metabolism and to correlate three NADP-linked enzyme systems to other measures of lipogenic capacity. Adult rats were reduced to 60% of their initial body weight by feeding first a protein free diet, followed by drastic food restriction. Control groups were killed at the beginning and end of the depletion period. The other animals were refed for 10 days diets containing 4.3, 13.0, or 38.7% of calories from protein. In addition three diets contained 20% corn oil. The effect of the dietary variables on the fate of intraperitoneally injected palmitate-1-^{14}C, hepatic and epididymal NADP-malate dehydrogenase, combine hexose monophosphate shunt dehydrogenase and NADP-isocitrate dehydrogenase activities were measured. Accumulation and fatty acid composition of hepatic and epididymal lipids were measured as indicators of changes in lipogenesis as well as acetate-1-^{14}C incorporation into fatty acids by the soluble supernatant of the liver.

Palmitate-1-^{14}C oxidation was not greatly influenced by changes in dietary constituents. Inclusion of fat into the

diets containing the high level of protein increased the oxidation rate during the four hours studied.

Large increases in the concentrations of arachidonic acid and the fatty acid C>20:4 in nonphospholipids occurred with the increase in protein from low to the intermediate or high level.

Hepatic lipogenesis was elevated over controls with the low fat, low protein diet, as indicated by both the fatty acid composition of the hepatic nonphospholipid fraction and acetate-1-^{14}C incorporation into fatty acids. Increasing dietary protein from the low to the intermediate level resulted in lower concentrations of palmitic, palmitoleic, and oleic acids and a decrease in the concentration of linoleic acid. Acetate-1-^{14}C incorporation into fatty acids was also decreased indicating a lower rate of lipogenesis. A further increase of protein to the high level had no significant effect on these measures of lipid synthesis.

Of the three hepatic NADP-linked enzyme systems studied, only NADP-malate dehydrogenase activity responded to the increase of protein from low to intermediate level in the low fat diets in the same manner as did acetate-1-^{14}C incorporation. However, when protein was further increased to the high level MDH activity was further depressed, whereas acetate-1-^{14}C incorporation was not affected. In contrast to MDH, hexose monophosphate shunt activity was increased with increasing levels of protein in the low fat diets.

Dietary fat depressed lipogenesis so that neither the combined percentages of palmitic, palmitoleic, and oleic acid nor acetate-1-^{14}C incorporation were significantly elevated over control values. Hepatic HMPD and MDH activities were also decreased by the addition of fat to the diet. Here as when the low fat diets were fed, malate dehydrogenase activity was decreased with increasing levels of protein. Hepatic HMPD activity was decreased when protein was raised from the intermediate to high level in the high fat diets.

Hepatic NADP-isocitrate dehydrogenase, though slightly elevated over control values in all refed animals, did not respond to dietary variables.

In contrast to the fatty acids of the hepatic nonphospholipid fraction, the fatty acids of epididymal lipid were not greatly influenced by the level of dietary protein. Palmitic, palmitoleic, and oleic acids were elevated over control values when the low fat diets were fed. The concentrations of these fatty acids were decreased with diets containing fat indicating decreased rate of lipogenesis. Hexose monophosphate shunt activity and NADP-malate dehydrogenase activity were higher in the adipose tissue than in the liver and were not affected by changes in dietary protein in the low fat diets. Inclusion of fat in the diet decreased MDH activity and this decrease was significant when protein was fed at the intermediate or high levels. HMPD activity was depressed by inclusion of fat into diets containing the intermediate or high level of protein,

but this influence was not seen when dietary protein was low.

NADP-isocitrate dehydrogenase was much lower in the adipose tissue than the hepatic tissue, and responded to changes in the refeeding diet. Increasing dietary protein from the intermediate level to the high level in the low fat diets decreased IDH activity. Inclusion of fat in diets with the intermediate level of protein also significantly decreased IDH activity.

Improvement in rate of gain, caloric intake, and relative concentration of epididymal lipid by inclusion of fat into low protein diets in the present experiment confirmed previous studies in this laboratory. However with increasing levels of protein these effects of fat were not seen. Increasing dietary protein from the low to the intermediate level improved food efficiency. Increasing dietary protein modified the hepatic nonphospholipid pattern so that it more nearly resembled that of the controls. This occurred simultaneously with a decrease in hepatic NPL and a decrease in acetate-1-^{14}C incorporation into fatty acids. The heightened activity of hexose monophosphate shunt dehydrogenases associated with high carbohydrate diets was dependent on the protein level of the diet.

These studies indicate that of the three enzymes studied, NADP-malate dehydrogenase is correlated closest with lipogenesis but the activity of this enzyme in hepatic tissue may more nearly reflect changes in dietary carbohydrate than

changes in lipogenesis. Hexose monophosphate shunt activity was not as well correlated with lipogenesis as was malate dehydrogenase activity. Isocitrate dehydrogenase activity did not seem to be correlated with lipogenesis in hepatic tissue, but the epididymal IDH activity was closely correlated to MDH activity and lipogenesis.

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ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Lotte Arnrich for her patience, understanding, and encouragement throughout the development of this problem and preparation of this dissertation. I wish to express my gratitude to Dr. Wilma Brewer and the Department of Foods and Nutrition for making this study possible.

I also wish to thank Jean Mitchell, Rita Taylor, and Daisy Chang for technical assistance and encouragement during the course of this project. Thanks are also due my committee for their time, encouragement, and friendship.

A special thanks goes to my husband, Wayne, for his patience, inspiration, and sense of humor.

APPENDIX

Table 32. Individual body weight gain, food intake, autopsy weight, respiratory ^{14}C, and weights epididymal tissue and lipid-experiment 1

Rat no.	Weight gains g	Food intake g	Autopsy weight g	Respiratory ^{14}C μc	Epididymal Tissue g	Epididymal Lipid g
			I			
78			509	4.44	9.0	6.39
138			494	3.27	7.0	4.27
139			500	4.13	5.8	4.11
140			470	3.35	5.5	3.72
141			460	3.61	5.5	4.10
142			482	3.74	5.1	3.34
143			484	4.00	6.3	4.69
144			472	3.57	8.1	5.91
145			462	3.06	8.7	5.67
146			474	2.90	8.1	4.84
Mean			481	3.61	6.9	4.70
			II			
7			280	1.44	0.6	0.04
25			288	1.91	1.2	0.36
30			283	1.89	1.7	0.84
41			288	1.58	1.4	0.52
43			275	2.12	0.8	0.08
51			289	1.94	1.4	0.46
89			300	1.92	2.2	0.13
97			286	1.65	1.2	0.32
116			300	2.08	3.5	0.20
124			294	2.88	1.8	0.69
Mean			288	1.94	1.6	0.36
			III			
9	53	154	327	1.91	2.0	0.99
10	61	165	335	1.29	5.8	3.51
59	53	174	336	2.37	2.5	1.23
74	68	199	360	1.89	3.5	2.05
82	55	158	332	1.84	5.6	3.56
87	56	163	338	1.94	3.7	2.26
96	69	211	344	1.75	3.4	1.72
108	61	180	340	1.86	3.5	1.96
119	54	159	338	2.11	2.8	1.64
122	68	182	354	2.88	3.5	1.90
Mean	60	175	340	1.98	3.6	2.08

Table 32. (Continued)

Rat no.	Weight gains g	Food intake g	Autopsy weight g	Respiratory ^{14}C μc	Epididymal Tissue g	Epididymal Lipid g
			IV			
5	66	155	333	1.94	3.7	2.41
11	80	168	335	3.42	4.6	2.74
19	67	155	335	2.44	3.5	2.30
57	67	152	346	1.64	2.8	1.47
61	69	156	356	2.07	4.0	2.53
88	69	149	352	2.16	4.6	2.79
90	67	157	346	1.84	4.3	2.99
92	65	145	348	2.31	3.1	1.89
100	76	164	348	2.50	2.9	1.42
120	81	181	364	2.79	3.3	1.71
Mean	71	158	346	2.31	3.7	2.22
			V			
15	67	136	335	2.06	2.6	1.32
31	48	120	330	1.94	2.8	1.56
44	75	146	356	2.02	2.5	1.45
48	63	133	340	1.94	5.3	3.60
68	74	155	360	1.77	4.5	3.06
81	76	154	354	2.36	3.0	1.61
85	67	137	348	2.26	2.5	1.33
98	80	153	352	1.87	3.8	2.38
123	84	164	362	1.97	5.4	3.74
125	56	122	342	1.97	4.6	2.89
Mean	69	142	348	2.02	3.7	2.29
			VI			
1	72	163	357	2.67	4.5	2.68
3	75	158	355	2.41	4.0	2.57
28	81	171	367	1.81	4.9	3.53
34	80	152	362	2.04	3.4	2.08
84	84	161	372	2.07	4.6	2.66
91	100	208	388	3.13	5.8	3.69
102	84	186	370	2.77	5.0	3.17
103	79	174	364	2.39	3.2	1.98
113	84	161	360	2.14	4.4	2.66
115	80	168	360	2.05	3.6	2.40
Mean	82	170	366	2.35	4.3	2.74

Table 32. (Continued)

Rat no.	Weight gains g	Food intake g	Autopsy weight g	Respiratory ^{14}C μc	Epididymal Tissue g	Epididymal Lipid g
			VII			
4	78	129	366	1.81	3.0	1.76
12	70	129	356	2.01	3.6	2.31
14	63	129	340	2.27	2.6	1.26
93	76	133	362	2.18	5.3	3.21
94	100	153	384	2.61	4.6	2.87
99	100	148	382	3.32	4.4	2.70
101	77	132	364	2.11	5.1	3.59
106	103	161	388	1.43	4.0	2.58
109	77	138	352	2.50	2.4	1.34
121	97	157	380	2.44	3.8	2.54
Mean	84	141	367	2.27	3.9	2.42
			VIII			
23	75	129	350	2.48	3.5	2.08
80	63	111	342	2.25	2.7	1.52
83	64	95	342	2.80	5.1	3.51
86	64	111	354	2.75	3.2	1.98
95	75	125	352	2.62	2.8	1.61
105	70	107	354	2.76	3.2	1.98
107	71	109	362	2.56	3.0	1.69
111	82	127	364	1.73	4.3	2.68
114	74	123	360	2.54	3.5	2.27
117	85	114	369	2.33	3.8	2.31
Mean	72	115	355	2.48	3.5	2.16

Table 33. Individual hepatic weights, hepatic lipids, and incorporation of palmitate-1-^{14}C into tissue lipids-experiment 1

Rat no.	Hepatic weights Tissue g	TL g	NPL g	PL g	Adipose TL μc	Hepatic TL μc	NPL μc	PL μc
				I				
78	13.4	.72	.37	.35	.368	.514	.246	.239
138	12.4	.62	.34	.31	.180	.701	.401	.286
139	12.4	.51	.12	.36	.094	.687	.181	.428
140	13.9	.65	.20	.46	.239	.639	.211	.301
141	11.6	.74	.33	.46	.201	.522	.199	.301
142	13.9	.61	.15	.44	.186	.533	.128	.413
143	12.8	.55	.14	.41	.089	.475	.145	.302
144	13.4	.57	.17	.35	.198	.431	.149	.268
145	11.9	.39	.13	.25	.349	.300	.202	.204
146	14.9	.54	.24	.37	.107	.420	.130	.264
Mean	13.1	.59	.22	.38	.201	.522	.199	.301
				II				
7	4.8	.16	.08	.09	.198	.900	.411	.876
25	6.6	.22	.06	.16	.820	.626	.121	.396
30	6.9	.22	.09	.11	.394	.440	.170	.222
41	7.4	.27	.13	.16	.394	.536	.240	.277
43	6.8	.24	.07	.14	.315	.440	.170	.426
51	7.1	.24	.09	.15	.394	.523	.169	.367
89	7.1	.21	.07	.14	.472	.373	.102	.223
97	7.2	.18	.06	.12	.383	.542	.159	.379
116	6.4	.23	.11	.14	.377	.234	.053	.150
124	6.7	.19	.05	.16	.195	.436	.079	.354
Mean	6.7	.22	.08	.14	.394	.523	.169	.367
				III				
9	7.8	.42	.27	.16	.218	.667	.356	.240
10	9.2	.56	.34	.21	1.377	.442	.217	.208
59	7.8	.74	.57	.19	.480	.397	.224	.164
74	8.4	.42	.21	.18	.436	.373	.182	.187
82	8.6	.63	.38	.20	.533	.965	.741	.295
87	9.2	.51	.27	.20	.292	.544	.297	.340
96	9.4	.67	.45	.19	.511	.498	.301	.164
108	9.0	.66	.42	.21	.549	.383	.155	.199
119	8.8	.32	.14	.19	.257	.349	.131	.208
122	9.6	.50	.30	.18	.442	.472	.201	.270
Mean	8.8	.54	.34	.19	.510	.509	.280	.228

Table 33. (Continued)

Rat no.	Hepatic weights: Tissue g	TL g	NPL g	PL g	Adipose TL μc	Hepatic: TL μc	NPL μc	PL μc
				IV				
5	8.6	.38	.19	.17	.750	.398	.138	.193
11	9.2	.54	.32	.24	.532	.590	.248	.356
19	8.8	.32	.12	.20	.408	.479	.172	.251
57	8.9	.29	.10	.17	.687	.398	.150	.258
61	9.2	.33	.16	.15	.509	.432	.229	.185
88	8.4	.48	.20	.27	.539	.519	.114	.331
90	8.3	.30	.10	.18	.631	.313	.120	.195
92	8.8	.24	.08	.15	.503	.418	.143	.250
100	8.7	.23	.07	.16	.249	.269	.058	.205
120	10.2	.35	.08	.26	.218	.373	.061	.280
Mean	8.9	.35	.14	.20	.503	.418	.143	.250
				V				
15	9.3	.35	.14	.23	.841	.496	.175	.670
31	9.3	.32	.12	.23	.731	.548	.163	.320
44	11.1	.34	.11	.26	.515	.402	.119	.331
48	9.2	.36	.11	.26	.687	.408	.124	.351
68	9.9	.44	.17	.24	.437	.405	.145	.252
81	10.4	.35	.14	.22	.407	.464	.159	.381
85	10.2	.39	.08	.32	.362	.375	.089	.328
98	9.2	.31	.07	.22	.430	.330	.088	.224
123	10.2	.29	.08	.20	.432	.248	.060	.186
125	8.8	.28	.06	.20	.310	.344	.072	.271
Mean	9.8	.34	.11	.24	.515	.402	.119	.331
				VI				
1	9.2	.59	.41	.21	.825	.440	.197	.231
3	9.8	.82	.64	.25	.703	.493	.237	.222
28	9.9	.63	.40	.25	.623	.420	.203	.218
34	10.3	.77	.45	.30	.441	.427	.164	.258
84	11.1	.68	.42	.27	.588	.378	.160	.233
91	12.0	1.10	.80	.33	.421	.450	.270	.304
102	7.8	.28	.13	.17	.544	.239	.063	.168
103	10.0	.74	.53	.18	.168	.299	.121	.204
113	10.0	.44	.32	.17	.585	.350	.166	.178
115	9.4	.47	.30	.17	.364	.294	.115	.214
Mean	9.9	.65	.44	.23	.527	.359	.170	.223

Table 33. (Continued)

Rat no.	Hepatic weights: Tissue g	TL g	NPL g	PL g	Adipose TL μc	Hepatic TL μc	NPL μc	PL μc
				VII				
4	9.7	.42	.26	.21	.508	.449	.219	.208
12	10.1	.46	.23	.23	.572	.501	.247	.206
14	9.1	.41	.21	.18	.343	.392	.151	.224
93	8.5	.33	.16	.16	.577	.399	.173	.178
94	10.6	.55	.25	.30	.203	.504	.212	.278
99	9.1	.42	.13	.28	.335	.441	.114	.339
101	9.9	.45	.16	.28	.380	.314	.071	.224
106	10.5	.44	.17	.28	.074	.213	.067	.138
109	9.6	.33	.16	.18	.142	.416	.150	.259
121	8.9	.44	.26	.21	.293	.293	.105	.189
Mean	9.6	.42	.20	.23	.343	.392	.151	.224
				VIII				
23	10.6	.41	.17	.22	.362	.445	.118	.288
80	9.4	.41	.14	.29	.219	.447	.120	.306
83	10.6	.43	.15	.30	.440	.549	.155	.399
86	9.4	.26	.07	.22	.333	.631	.060	.246
95	9.5	.39	.16	.23	.354	.447	.224	.235
105	9.4	.41	.10	.29	.290	.515	.130	.377
107	9.7	.30	.08	.21	.493	.308	.072	.241
111	11.6	.51	.22	.33	.498	.474	.163	.356
114	9.5	.35	.10	.26	.162	.299	.082	.199
117	9.8	.30	.10	.24	.468	.333	.060	.231

Table 34. Individual body weight gain, food intake, autopsy weight, and tissue weights-experiment 2

Rat no.	Weight gains g	Food intake g	Autopsy weight g	Tissue weights Adipose g	Tissue weights Hepatic g
		I			
113			489	5.3	10.5
114			480	8.2	13.0
115			480	5.4	12.9
116			482	5.7	12.1
Mean			482	6.1	12.1
		II			
86			292	0.7	6.1
93			298	2.6	5.8
94			297	2.1	6.7
99			300	3.6	5.9
101			296	1.4	4.9
102			277	0.6	4.9
107			299	1.5	6.5
110			292	1.6	6.3
Mean			294	1.8	6.1
		III			
5	74	190	350	2.5	10.0
9	68	200	361	2.7	9.6
13	82	195	361	3.9	10.5
20	83	204	361	3.2	8.9
25	67	169	333	3.3	10.3
34	69	199	354	2.7	9.0
41	57	167	341	2.5	9.0
42	57	153	340	2.4	9.5
51	58	166	333	1.8	8.8
58	59	179	342	3.7	8.7
66	67	186	350	3.0	9.2
67	44	149	327	1.7	8.7
74	65	169	341	1.8	9.8
81	89	219	366	3.1	11.1
89	69	219	360	1.9	11.0
Mean	67	184	348	2.7	9.6

Table 34. (Continued)

Rat no.	Weight gains g	Food intake g	Autopsy weight g	Tissue weights Adipose g	Tissue weights Hepatic g
		IV			
2	74	162	350	2.9	8.5
3	79	185	362	2.9	9.5
11	74	173	354	2.4	9.4
18	69	161	353	2.5	8.4
27	77	169	363	3.1	9.0
38	71	158	355	3.4	9.3
47	70	154	351	2.6	8.9
48	83	179	359	2.1	9.4
55	74	177	351	2.1	8.7
56	75	164	359	2.9	8.5
64	56	152	342	3.1	8.0
71	84	167	363	3.0	9.6
76	88	182	360	2.1	10.2
91	92	192	369	2.1	8.2
98	79	193	360	2.6	8.5
Mean	76	171	357	2.6	8.9
		V			
1	48	117	343	2.5	9.7
6	75	142	354	2.9	10.0
10	69	152	356	2.8	9.9
26	72	155	356	2.8	9.6
31	62	135	341	2.4	9.1
33	80	156	362	2.7	12.0
49	89	151	358	2.2	10.1
52	89	163	361	2.1	10.1
57	75	149	358	3.4	9.8
60	67	146	353	3.5	9.1
61	76	151	354	1.4	9.3
73	63	148	343	1.7	9.7
75	74	157	358	2.5	9.6
90	71	156	353	2.4	8.4
95	72	156	344	2.2	8.9
Mean	72	149	353	2.5	9.7
		VI			
8	81	183	364	3.7	10.3
15	105	200	381	4.3	9.6
19	86	179	371	4.9	12.8
21	72	151	360	2.7	9.6
35	80	164	358	4.4	10.6
37	65	155	357	3.9	9.2

Table 34. (Continued)

Rat no.	Weight gains g	Food intake g	Autopsy weight g	Tissue weights Adipose g	Hepatic g
39	88	152	373	3.8	9.8
44	63	145	347	2.2	9.3
46	70	152	360	4.1	10.2
63	80	170	363	2.9	8.4
65	73	162	338	2.3	9.9
70	83	189	373	1.4	9.0
82	92	190	373	4.7	11.1
84	72	167	355	4.8	9.3
96	63	150	348	2.6	9.1
Mean	78	167	361	3.5	9.9
		VII			
14	95	159	377	2.7	9.3
16	86	163	371	2.9	9.8
17	91	149	372	3.8	9.7
22	74	139	357	2.7	8.7
28	93	156	371	3.4	9.7
43	86	147	360	2.7	11.2
45	92	159	371	3.1	9.9
54	92	164	381	4.8	9.3
69	64	133	343	2.4	7.7
78	90	150	370	3.4	10.2
79	92	153	377	4.1	9.8
80	89	156	373	6.3	9.3
85	102	168	386	4.2	10.8
87	60	128	348	3.7	9.6
88	87	156	372	3.4	9.0
Mean	86	152	369	3.6	9.6
		VIII			
7	66	120	356	4.0	9.4
12	80	115	359	3.3	9.3
23	51	108	330	1.8	9.6
29	74	118	350	2.7	9.4
30	55	84	343	3.1	8.6
32	79	120	362	3.4	10.4
36	76	120	362	2.8	8.2
40	89	123	372	3.9	9.1
53	85	135	363	4.0	9.6
59	55	107	347	3.5	8.3
72	68	130	332	2.3	10.0
83	73	133	356	3.3	9.5
97	70	131	359	2.2	10.2
100	78	136	347	2.2	10.0
Mean	70	120	352	3.0	9.4

Table 35. NADP-linked enzyme systems-experiment 2

Rat no.	Hepatic MDH	Hepatic HMPD	Hepatic IDH	Epididymal MDH	Epididymal HMPD	Epididymal IDH	Cytosol protein Liver g	Cytosol protein Adipose g
	ΔOD/100 mg protein							
				I				
113	.52		12.1	.24		.25	1.54	.10
114	1.29		10.2	.10		.20	1.77	.10
115	.66		8.1	.22		.57	1.97	.11
116	.51		8.4	.55		.62	1.56	.06
Mean	.74		9.7	.28		.41	1.71	.09
				III				
5	8.29	7.40		18.67	10.67		1.45	.11
13	9.65	5.87		45.42	29.62		1.48	.10
20	2.13	7.46		73.48	30.62		1.13	.07
34	6.02	3.38	11.7	30.53	20.99	1.67	1.63	.10
25	4.92	6.65	16.1	14.46	4.34	1.40	1.44	.07
42	4.12	6.48	17.7	15.66	10.44	2.56	1.48	.06
41	4.82	5.39	14.4	22.66	13.39	1.93	1.58	.10
51	4.36	3.70	14.2	33.02	5.83	1.73	1.22	.05
58	5.87	7.30	13.2	6.58	4.11	.97	1.19	.12
67	2.80	2.72		27.71	13.04		1.29	.06
Mean	5.30	5.64	14.6	28.82	14.30	1.71	1.39	.08
				IV				
3	6.06	7.91		94.91	34.51		1.24	.07
11	5.90	4.16		57.72	31.27		1.37	.04
2	1.65	3.38		99.97	33.79		1.21	.07
18	2.82	9.55	24.0	46.96	44.03	3.43	1.28	.07
27	1.18	8.32	13.3	9.45	12.03	1.14	1.61	.12
55	1.93	7.97	23.9	24.02	7.58	.94	1.19	.08
64	2.13	6.89	17.2	34.52	24.37	5.28	1.45	.05
48	3.68	10.25	17.5	17.07	11.73	1.92	1.52	.09
76	2.29	5.18		27.77	5.21		1.49	.06
56	3.07	7.07	19.2	45.84	22.70	2.55	1.37	.09
Mean	3.07	7.07	19.2	45.82	22.72	2.54	1.37	.07
				V				
10	2.48	3.26		97.08	43.10		1.41	.03
6	2.94	7.64		29.83	8.70		1.26	.06
33	1.77	5.77		33.12	4.90		1.80	.07
31	.59	3.86	18.2	17.57	34.77	2.68	1.34	.05
24	1.34	6.30	18.1	17.17	18.76	1.49	1.49	.07
49	2.56	15.75	2.4	14.72	8.61	1.42	1.52	.07
57	.82	8.82	14.4	34.75	25.79	2.77	1.46	.07
61	2.80	11.19	19.6	44.69	23.43	3.25	1.00	.05
52	2.36	8.54		75.09	16.55		1.44	.06
60	2.67	9.90	17.0	28.17	18.11	1.81	1.27	.06
Mean	2.03	8.10	14.9	39.22	20.27	1.34	1.40	.06

Table 35. (Continued)

Rat no.	Hepatic MDH	Hepatic HMPD	Hepatic IDH	Epididymal MDH	Epididymal HMPD	Epididymal IDH	Cytosol protein Liver g	Cytosol protein Adipose g
	ΔOD/100 mg protein							
				VI				
8	1.04	.82		18.06	15.48		1.82	.03
15	3.17	2.51		15.23	10.99		1.20	.06
19	2.62	1.47		18.06	31.35		1.57	.07
21	1.40	2.03	16.8	9.99	13.56	.93	1.28	.04
35	1.88	1.94	10.0	10.16	7.27	.39	1.91	.11
44	2.43	6.15	12.2	11.26	109.39	.87	1.48	.05
37	1.37	1.60		2.82	26.57		1.32	.09
46	1.38	1.46	22.0	4.88	12.80	.54	1.31	.08
65	3.44	2.38	9.5	18.03	8.47	.74	1.51	.07
63	2.17	2.28	16.9	21.93	15.41	2.44	1.71	.13
Mean	2.09	2.26	14.6	13.04	25.13	.98	1.51	.07
				VII				
14	1.13	2.32		4.49	13.94		1.51	.04
16	1.99	2.78		13.01	3.45		1.40	.08
17	.54	1.45	11.8	2.13	8.86	.57	1.66	.08
22	.50	1.13		9.14	5.62		1.59	.07
28	.52	2.66	20.3	7.03	7.98	.54	1.35	.08
39	.92	2.41	15.7	4.71	2.36	1.66	1.56	.07
43	1.47	5.40	19.2	6.76	10.14	.48	1.70	.07
45	.71	1.12	13.2	4.89	3.43	2.10	1.70	.07
54	.46	2.83	14.0	11.75	11.43	1.04	1.30	.10
69	.93	1.99		5.53	1.84		1.40	.09
Mean	.92	2.41	15.7	6.94	6.90	1.06	1.52	.08
				VIII				
7	.41	1.63	16.0	2.74	4.21	.60	1.47	.07
12	.38	1.14		5.05	3.91		1.32	.06
29	.48	1.23	17.5	2.18	4.04	3.07	1.46	.05
23	.24	.67	3.2	5.32	5.62	.68	1.64	.05
30	1.00	2.60	17.2	2.49	2.04	.63	1.50	.08
32	.26	2.00	17.7	7.42	3.30		1.55	.07
40	.31	1.22		3.25	2.47		1.64	.11
36	.42	1.11		4.97	2.63		1.44	.07
59	.15	.93	13.9	2.98	4.38	.77	1.29	.07
53	.77	.89		2.85	4.95	.58	1.68	.10
Mean	.44	1.34	14.2	3.92	3.76	1.06	1.50	.07

Table 36. Acetate-1-^{14}C incorporation into fatty acids per mg protein-experiment 2

Rat no.	cpm x 10^3	Cytosol protein g/liver	Rat no.	cpm x 10^3	Cytosol protein g/liver
	I			II	
113	3.86	1.61	86	2.31	.71
114	5.88	1.96	93	7.24	1.06
115	.42	1.88	94	2.97	.82
116	6.40	1.88	99	1.68	.53
			101	1.41	.77
			102	2.51	.89
			107	2.10	1.07
			110	1.68	.90
Mean	3.27	1.83	Mean	2.78	.84
	III			IV	
51	7.43	.60	48	40.80	.96
58	25.22	1.24	50	6.95	1.16
66	24.04	1.10	56	4.67	1.29
67	11.57	1.21	64	4.73	1.33
74	23.49	1.34	71	12.07	1.38
81	35.90	1.53	76	7.93	1.46
89	23.37	.72	91	11.97	1.09
92	26.64	1.05	98	1.33	1.02
Mean	19.56	1.08	Mean	10.31	1.21
	V			VI	
52	8.18	1.58	46	1.57	1.25
57	20.84	.96	63	2.30	.96
60	6.23	1.46	65	.60	1.22
61	14.72	1.45	68	5.56	1.23
73	24.68	1.45	70	2.81	.96
75	2.25	1.31	82	12.52	1.33
90	4.73	1.33	84	6.74	1.28
95	7.61	1.34	96	19.25	1.44
Mean	11.15	1.36	Mean	6.42	1.21
	VII			VIII	
54	1.49	1.41	36	.56	1.29
69	3.35	1.18	53	3.19	1.40
78	2.05	1.16	59	1.06	1.26
79	3.04	1.27	72	1.81	1.45
80	2.17	1.34	77	1.82	1.29
85	5.60	1.29	83	1.39	1.27
87	.72	1.23	97	.89	1.40
88	2.07	1.32	100	.98	1.52
Mean	2.56	1.28	Mean	1.46	1.36